

5 March 1981

Pope John Paul II in Hiroshima

The Pope's visit last week to non-Christian Japan showed how well this indefatigable traveller can tune his speeches to his audience. With the faithful thin on the ground, general issues took precedence over those that domestically occupy his Church. The result was a more open discussion of some perplexing problems than in the Philippines the week before. And nobody should complain that the Pope followed other statesmen to Hiroshima for a major speech on the problem of nuclear war. The melodrama of the site ensures that some notice will be taken of what is said. Not surprisingly, the Pope's discussion was a good deal more perceptive than many that have preceded it. The visit also provided him with an opportunity to pat on the back one of the two sponsors of the occasion, the United Nations University. The university's new rector, the Indonesian Soedjatmoko, seems well placed to help the university make a mark in its chosen field of interest, the development of developing countries.

The danger facing those who make speeches at Hiroshima is that of following the same hackneyed theme. Nuclear weapons are awful things, and the product of scientific research, from which it follows that the sharpest dilemma is the Promethean dilemma. The Pope fortunately had the wit to acknowledge that this task has political as well as ethical or moral dimensions. However powerful may be people's collective wish that nuclear war should not break out, their political differences ensure that the danger will remain alive. It would be asking too much to accept the argument, for which there is some justification, that only the balance of terror has kept the peace these past thirty years. But the implication of his remarks (at Nagasaki) that the United Nations must more powerfully assume a peacekeeping role is needlessly unearthly; the United Nations is a place where governments with different interests meet, as often to declare their differences as to resolve them. Although the United Nations has from time to time passed high-sounding resolutions on the need for "general and complete disarmament" (known as "GCD" in the trade) the painfully slow processes of the Committee on Disarmament shows what problems lie ahead.

Fortunately, not all problems are such inherently gloomy problems. Although the theme of the Pope's speech last week was "the ethical problems of the technological society", he reminded those who heard him that "we all greatly benefit" from the application of science and technology. In the past few gloomy years, optimism on this score seems to have melted surreptitiously away, not so much from anxiety about war as because of the faltering of the industrial economies and the predictable but nonetheless bitter failure of efforts such as the United Nations conference on technology and development two years ago. But can it be that the promise of science and technology for the improvement of the human condition, widely believed only a few years ago, has evaporated overnight? Is it not more likely that people have become discouraged? And, in these circumstances, may it not be especially valuable that vaguely incongruous figures like the Pope should go about reminding people that the promise is still there to be captured?

But how? At Hiroshima, the Pope said that the need was for a "moral about-face". Translated into the language of everyday politics, the implication is that governments must become more serious in their consideration of documents such as the report of the Brandt Commission on the problems of contemporary development, more imaginative in their management of domestic wealth and liberty and more courageous in their handling of dealings with each other. But, in the last resort, governments —

even illiberal governments — are merely the products of their people. Is the practical message of what the Pope was saying last week about the moral responsibility of the scientific community that the time has come when governments should be reminded again, as they have been *ad nauseam* in earlier decades since the Second World War, that the time has come to start solving problems, not to be mesmerized by them?

Ructions in space

The European Space Agency is using unaccustomed undiplomatic language in its attempts to persuade the new United States Administration not to force the cancellation of the joint venture to put a pair of spacecraft into polar orbits about the Sun. Rumours that this might be one of the consequences of the cut in the budget of the National Aeronautics and Space Administration have been circulating in Washington for several days (see *Nature* 26 February, p.738). The European reaction to the news that the rumours are true has been understandably tetchy. Europe, the European Space Agency is saying, has been led up the garden path, has been induced to lavish a substantial part of the meagre funds available for planetary exploration on a project whose value will now be much reduced (see page 3), and which may even have to be abandoned altogether. By asking that member states should send their diplomats into action in Washington, the European Space Agency has broken new ground in the foreign relations of multilateral agencies like itself. The bad temper in its public statement on the problem suggests that good relations may be at an end.

Luckily, that time may not yet have arrived. There remains a chance that the American half of the solar polar mission will be restored in the budget to be published on 10 March. More to the point, there is every chance that the United States Congress, still not quite certain what to make of President Reagan and his well-intentioned but draconian fiscal policies, will write back into the budget some of the items the President is planning to strike out. (And then, of course, it is always possible that the White House might decide not to spend the money, anyway.) In short, it is too soon to be supposing that the solar polar experiment is a washout. What has happened is more subtle but also more important.

Domestically, the United States space programme is in a mess. For the past three years, the space shuttle, the machine intended as the launching vehicle both for commercial and scientific spacecraft, has been in trouble. It is already two years late, and further difficulties may come to light in the critical months ahead. Yet it is too late to complain that the United States should have embarked on a quite different programme of development ten years ago, when the Apollo programme ended. Willynilly, the shuttle is the only sufficiently versatile launching system in sight. If its use is further delayed, the consequences will be more serious than the cancellation of the American half of the solar polar project. The European half may be without a launcher as well, while a whole string of communications satellites will have to find their way into synchronous orbits by quite different means. Worse still, for the scientific community, the Large Space Telescope in which the European Space Agency also has a substantial stake and which the United States Administration has so far (to its credit) kept free from cuts, will also be in jeopardy. Bad temper is, in the circumstances, inappropriate.

Looking further ahead, however, the European Space Agency



is right to be alarmed. In the past few years, it has dawned on European governments that they must have access to some way of launching spacecraft of all kinds. The chance to join the shuttle project as a partner has however gone. The European launcher Ariane may have a future, if only as an insurance policy against further delay of the shuttle and as an assurance of independence, but is not the ideal way of meeting the need. The space agency itself, now under new management, has a poor public reputation stemming at least in part from its bureaucratic ways and from the way in which member governments appear to regard it as their first objective to ensure that their contributions are requited by contracts placed with national companies. Many scientists concerned to put scientific experiments into space have found it easier to work with the American agency than with that in Paris. The first lesson to be learned in Paris from the threatened cancellation of the American half of the solar polar project is that it should put its own house in order. Only then will it be able to take the lead in making sure that European governments collectively have a view of their future role in this important field.

The European Space Agency would at the same time do well to draw a wider lesson from what is going on in Washington. The space budget is being cut not only because of the technical problems with the shuttle but also because expenditure of this kind is not immediately productive. In the long run, it is better for Europe that the American economy should be healthy (free from inflation) and productive than that funds should be found for the second half of the solar polar project. And, it would be folly if the growing European enthusiasm for space projects were further to undermine the economic health of Western Europe.

Questions answered

The British predilection for studying the machinery for administering publicly supported science has burst out again, this time in the House of Lords (*Nature* 26 February, p.741). British governments seem constitutionally unable to decide for themselves what sensible steps to take, and usually set up *ad hoc* committees to tell them what to do. The select committee of the House of Lords is an unexpected but a better way of tackling the problem. The committee has also taken the unusual step of inviting written evidence from anybody, and the specific questions it has asked show that its inquiry may well be radical. What follows may help to start the ball rolling.

The most important, because the most novel, group of questions is that labelled machinery of science. Could the relationship between the British government and the scientific community be improved and, if so, how? The obvious difficulty is that, formally, there is no such thing as a scientific community. There are a few organizations, the Royal Society chief among them, which are sufficiently diverse to be consulted as if they were representative, and so eminent that the advice they give is rarely foolish. However radically the House of Lords committee tackles its inquiry, these influences on government will (and should) continue. Moreover, it would be fruitless to engage an army of constitutional lawyers to devise some way in which the wider scientific community could speak with even greater representative authority. The truth is that opinions within the scientific community differ about all the questions the committee has asked as well as about more specific problems.

What the British government needs is some means by which it can sense the diversity of scientific opinion on contentious issues, forming in the process a judgement about which views should be taken seriously, and at the same time making more effective use of the imagination and willingness to help of people working in laboratories of all kinds. The mechanism required is more like a lightning-rod than a forum. Whether it should be more like the United States President's Science Advisory Committee than the British Advisory Council on Scientific Policy of the 1950s is less important than that it should be accessible to all who want to tell it something.

Given such a device for drawing attention to important problems, some of the select committee's questions would be

answered. Thus, while no amount of organization can ensure that there is sufficient communication between those responsible for the administration of science in industry, research councils and government, common experience shows that public servants respond diligently to well-considered public complaint or even to constructive suggestion. To be effective over the whole field the select committee has mapped out, a committee of this kind would have to be adequately serviced — previous essays in this direction have been less than successful because they have been run on a shoestring. The terms of reference of the existing Advisory Council on Applied Research and Development are too narrow to do what is now required, and the council seems shy of tackling questions of how well government machinery is working. It is, however, essential that any such organization should be independent of particular government departments. The Council for Scientific Policy of the 1960s, which did some useful work, was too dependent on the Department of Education and Science to be effective. The need now is for a channel for communication free from departmental interests. The House of Lords is well placed to decide how such a committee should be plugged into the government machine. Direct responsibility to the Prime Minister would be the constitutional ideal but would make a mountain out of a molehill. A connection with the Treasury would not offend the lawyers and could be fun. Otherwise, the best choice would be a return to the 1960s, when the Lord President of the Council was responsible for the Council on Scientific Policy.

Other questions on the list from the House of Lords bear on the issue of the Rothschild customer-contractor principle. In spite of the mixed experience of the past decade, it would be wrong now to give up the notion that government departments should be equipped to decide scientific questions relevant to their own work, and to commission their own research. But the system in which departments were required to appoint chief scientists to manage their scientific affairs has not always worked well. Some of those appointed have not been up to the job, few of them have been in their posts long enough to learn what to do and none of them has been paid enough. However, there can be no general rule for deciding how much money should be spent by departments and how much by research councils. Research councils should have enough money to discharge their responsibilities properly, and government departments should spend money on research in the expectation of winning tangible benefit as a result. Some of the imperfections of the present system, and especially the difficulties which have arisen in the financing of applied research in which no government has a commanding interest, would not exist if the arrangements for dividing the science budget between those dependent on it took more account of the scientific merits of their different programmes.

These are the easy questions. The committee's question about the adequacy of the government's own scientific manpower is much more difficult. What should be done about the scientific civil service? And what should be done to equip the British civil service as a whole to grapple with modern problems? The British government is at present too directly involved in research in its own establishments (shockingly so in defence research). If the committee does more than skate round the manpower problem, it will recommend that the government reduce its own research effort drastically (but provide better careers for those who remain) and also tackle seriously the recruitment of scientists in mid-career to the civil service proper. The present system merely perpetuates amateurism and ensures inept advice.

The hardest question of all, however, is missing from the list circulated by the House of Lords. What is to be done about defence research? Only two weeks ago (*Nature* 19 February), the European Commission was pointing out that defence research is a larger proportion of all research in the United Kingdom than elsewhere in the European Community. Outsiders have no means of telling how necessary this vast enterprise may be, although there is every reason to believe that the huge defence laboratories could be used more effectively to foster industrial change without hazarding national defence. The select committee should think of adding this topic to its agenda.

Open row about joint space project

European space agency plans protest

Washington

Western European nations have agreed to organize a top-level protest to the US State Department at the proposal of the National Aeronautics and Space Administration (NASA) to withdraw its spacecraft from the International Solar Polar Mission.

The mission, under which two spacecraft would be flown simultaneously over opposite poles of the Sun, is being organized jointly by NASA and the European Space Agency. Originally each agency was to have supplied one of the spacecraft, the two being launched from the space shuttle in 1985. NASA has now decided to absorb some of the massive cuts in its space science programme required by the Reagan Administration by terminating the work on its own spacecraft. It would still provide support facilities for the European spacecraft if the European Space Agency decided to press on alone.

The European agency issued a strongly-worded statement last week saying that it considered NASA's actions to be a unilateral infringement of the memorandum of understanding signed between the two countries, and warning that it could have severe consequences for future co-operation in space research between Europe and the United States. Now several countries, including France, West Germany, Italy and the United Kingdom, have agreed to make a joint protest to the State Department, hoping that NASA can be persuaded to change its mind. Without the participation of the US spacecraft, which would have provided the first opportunity to study a star at close hand, the value of the mission will be severely limited.

Within NASA, however, officials have little hope that the proposed cuts will be reversed, since next to the space shuttle programme NASA's top priority has been to keep intact its two main space science projects, the space telescope and the Galileo mission to Jupiter.

NASA officials have stressed the importance they attach to maintaining links with European colleagues, as both acting deputy director Anthony Calio and the director of the Goddard Space Flight Center, Dr A. Thomas Young, pointed out when Mrs Margaret Thatcher visited the centre during her visit to Washington last week.

However, NASA has had to take some hard decisions itself, and apparently decided that the cancellation of its share in the solar mission was the least of several evils. On the other hand, the deferral of the

gamma-ray observatory and of the Venus Orbiting Imaging Radar have been demanded by the White House.

NASA has now been asked by the Office of Management and Budget (OMB) to make even more cuts in its next year's budget, following the discovery that the size of the required federal budget cut had been underestimated. Although it is not known where these most recent cuts will fall, one rumour is that funding for astrophysics experiments on the second Spacelab will be withdrawn, a decision which would particularly hit British scientists.

When rumours began to circulate in Washington last month that OMB was proposing to scrap Galileo, West Germany — which is developing several of the instruments to be flown on both the orbiter and the probe and is contributing about \$50 million to the cost of the mission — protested strongly to Secretary of State Alexander Haig. Mr Haig's subsequent intervention with OMB is said to have contributed significantly to the decision not to terminate the project.

No mention was made at that time of a possible threat to the solar polar mission. NASA was given its total required budget cut but left to decide where the cuts would fall. Cancelling the solar polar mission spacecraft would save between \$250 and \$300 million total costs over the next eight or nine years.

Gloom at prospect of cancellation

Officials at the European Space Agency (ESA) are too upset by the decision by the National Aeronautics and Space Administration (NASA) to ditch its solar-polar spacecraft to talk about contingency plans, preferring to await the results of diplomatic efforts to get the decision reversed before 10 March when the federal budget goes to Congress and after which changes cannot be made. Nevertheless it is almost certain that, if it has to, ESA will go it alone, having already invested too much in the mission to abandon it.

The European Space Agency council meets this Thursday (5 March) to decide on its reaction to the US moves. There is still a chance that some of the US instrumentation could be transferred to the European craft, although European experiments on the US craft would be given higher priority. The agency could equally well decide that, without full US participation, the mission is not worth pursuing, a decision which would severely disrupt its space science budget.

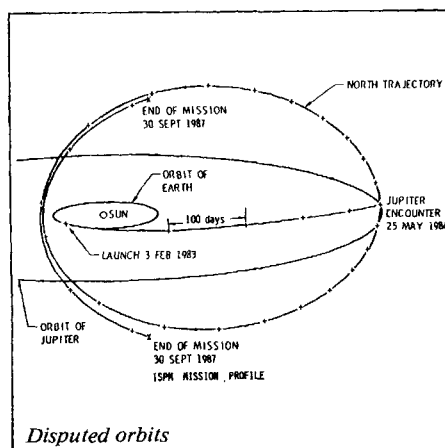
NASA's final proposals are expected to be announced next week, when Mr Reagan gives Congress a detailed list of his proposed budget reductions. There will undoubtedly be some opposition in Congress if the mission remains among the proposed cuts. Senator Jack Schmitt, an ex-astronaut and now chairman of the science and space subcommittee of the Senate Commerce Committee, said last week that he was very concerned that the decision could jeopardize future cooperation, particularly at a time when Europe seemed to be moving ahead of the United States in some areas of space technology.

But, as a loyal supporter of Mr Reagan, Mr Schmitt said he also acknowledged that there was a need to make substantial cuts in federal spending to restimulate the economy — and that space science, together with other research areas, would have to accept its fair share. **David Dickson**

The International Solar Polar Mission was originally conceived as a two-spacecraft mission that would observe dynamic and variable phenomena at high solar latitudes. The spacecraft's journey round Jupiter would also provide the first opportunity for spatially and temporally resolved measurements of Jupiter's magnetosphere. Some of the experiments to be flown on the two spacecraft would be identical but others would complement each other.

A decision to abandon the NASA spacecraft would mean that spatial and temporal resolution could be achieved less adequately only by comparison with simultaneous observations from the Earth. In all probability, it would also mean that the NASA spacecraft's ability to make optical measurements of the Sun's surface — unlike the ESA spacecraft, it has a pointing device — would be lost.

Each spacecraft is to house some of the other partner's experiments. It is thought unlikely, because of commitments already made, that the experiments to be flown on the ESA spacecraft could be changed at this late stage. Fortunately NASA has said that it will continue support of its experiments to be flown on ESA's craft. That, however, begs questions of the



future of European experiments for the NASA vehicle. According to ESA, work on those experiments is already well advanced and substantial sums of money, which will have been spent in vain if NASA's decision stands, have already been committed. The total cost to completion of the two spacecraft is an estimated \$140 million, \$100 million of which is already accounted for.

Judy Redfearn

European fusion

JET's ambition

JET, the joint European tokamak nuclear fusion experiment, may cost another 400 million European units of account (EUA) (£216 million) if first the JET Council — which oversees the project — and then the European Commission agree. The money would be spent over five years, beginning in 1982, and would extend JET to its full design performance, including bulk ignition of a deuterium-tritium plasma. "If they want the results, they'll have to pay" said Dr Hans-Otto Wüster, director of JET, of the European governments last week.

The original cost of basic JET was 185 million EUA at January 1977 prices. Inflation in Britain, coupled with the strength of the pound sterling and a serious underestimate of the cost of diagnostic equipment, has pushed that basic price to something nearer 300 million EUA; but even so the proposed extension would more than double the total cost. Dr Wüster, however, points out that the extra cost is only twice the annual budget of the CERN laboratory in Geneva.

The rush to extend JET is occasioned by two factors. First, machines like JET seem to have a better chance of reaching ignition, the nuclear burning of the plasma, than when they were designed in the early 1970s. Second, the European Council of Ministers has asked for a proposal for fusion research in Europe for the next five-year period, 1982–86, by 1 July this year.

Competition with the United States is also in mind. Princeton's Tokamak Fusion Test Reactor is due to begin experiments in 1982, and the somewhat larger JET in early

1983. Princeton is being pushed hard by the US Department of Energy to try ignition early. If all went well, JET's initial non-nuclear plasma experiments would then look pretty tame. On the other hand, if either laboratory is too hasty in introducing radioactive tritium, repeating early experiments will be very difficult, requiring remote handling and radiation protection.

Important plasma physics problems remain to be solved. Present optimism arises because two predicted limits on plasma containment have not been observed in existing small tokamaks. "Trapped particle instabilities" were expected to set in at temperatures above 30 million K, but the smaller Princeton tokamak has reached 70 million K without seeing them. And the ratio of plasma pressure to magnetic pressure was thought to be limited to about 1 per cent, but values of 8–9 per cent have been reached. While experimentally encouraging, these results are theoretically puzzling, so the JET and Tokamak Fusion Test Reactor regions may still hold surprises.

Dr Wüster is therefore cautious, offering 3–4 years of plasma at JET before attempting ignition around 1987. But the bulk of the spending on "extended performance" would have to be undertaken in 1982–86. In January, the JET Council gave Wüster a 400 million EUA guideline for the extended performance, and asked him to say what he could do with that. If, this month, his proposals are adopted, it will be for the European Commission to consider whether to include it as part of the 1982–86 research programme.

Conflicts between the interests of the national laboratories and money-hungry JET seem likely to be resolved at JET Council level; but the commission must also test the interest of governments, for which reason it has set up a new committee — the Consultative Committee for Fusion Programmes — which will be dominated by the representatives of government departments from whose coffers the money must ultimately be found.

The commission has also established an expert panel, the European Fusion Review Panel, to make recommendations for a long-term programme, beyond the present generation of machines. This panel will also report to the commission before July, in time to influence the final commission proposal.

One question to be dealt with is whether Europe is wise to commit itself so heavily to one design of fusion device, the tokamak. In the United States, there is also substantial work on the potentially simpler magnetic mirror confinement and inertial confinement. Wüster says that it makes sense to push ahead with the device that will most quickly yield ignition, so as to win practical experience of the problems of operating fusion reactors of all types. We shall see whether the European Fusion Review Panel agrees with him.

Robert Walgate

United States budget

More cuts

Washington

True to its political convictions, when full details of its proposed budget cuts are announced next week, the Reagan Administration is expected to eliminate virtually all the federally sponsored programmes initiated by President Carter to stimulate innovation in private industry.

President Carter's initiatives were the result of a broad-ranging, eighteen-month study largely instigated by the Office of Science and Technology Policy. Recommendations were made by eight independent advisory committees, and submitted to the White House with proposals from individual federal agencies.

From these, a package of 32 proposals was eventually accepted and announced by Mr Carter in November 1979. Although criticized at the time for not going far enough, it was generally agreed that the proposals made up a modest set of experimental and exploratory approaches aimed at bringing industry, government and the universities closer together.

The interventionist approach which the new programmes embodied, however, has found little sympathy with the new Administration, which sees its principal strategy for stimulating innovation as improving the financial incentives for investment, not direct federal participation.

The Administration will therefore be expected to drop support for any future co-operative generic technology centres, even though specific legislation setting up these centres was approved by Congress last year, and \$5 million allocated to their support (*Nature* 286, 195; 1980). From the three centres initially proposed, only one, in Detroit, is likely to survive as plans were agreed before the election, although even this is uncertain since it will depend on the centre's ability to raise matching funds from industry.

Other projects previously under development in the Commerce Department and now expected to be phased out include proposals to establish state-based Corporations for Innovation Development to help entrepreneurs gain access to investment capital, and the new Office of Technology Strategy and Evaluation.

It is also rumoured that the new Administration may not seek a successor to Dr Baruch, who as Assistant Secretary for Productivity Technology and Innovation was responsible for science and technology programmes within the department. This would be opposed by Congress, which spent much time last year discussing the Carter Administration's initiatives, and supported their general thrust.

Another of Mr Carter's initiatives which will be overruled is the Co-operative Automotive Research Project (CARP), originally proposed by Transportation Secretary Mr Brock Adams as a means of

JET upgraded

The extension of JET to full design performance would involve increased magnetic confinement fields, additional heating of the plasma, facilities for storing and handling tritium, and remote handling equipment for experiments. Specifically, the peak toroidal field coil power would be increased from 250 MW to 380 MW; total magnetic field at plasma centre 27.7 kG to 34.5 kG; plasma current from 3.8 MA to 4.8 MA; and additional heating power from 4–10 MW to 25 MW.

Robert Walgate

generating joint government and industry support for long-term research, and already approved by Congress with a budget of \$12 million for the first year. In his budget proposals of two weeks ago, Mr Reagan said that "federal financing of long-term research to benefit a particular industry is an inappropriate allocation of federal funds". He is proposing to rescind the full amount appropriated by Congress for the current year, and to terminate the whole programme.

At the National Science Foundation, schemes for encouraging greater technological innovation in small businesses, and for forging closer links between industry and universities, will remain in force, but will not get the substantial increase in funding that the Carter Administration had promised as part of its innovation package.

Support for small industries innovation, for example, was to have been almost doubled, from \$7.5 million to \$14.5 million next year, following its earlier success, but will now be cut back. So too will increased funds for engineering education, but the proposed 20 per cent increase for engineering research is likely to remain.

In contrast with the cuts being proposed in measures which would increase federal involvement in the innovation process, other steps initiated by Mr Carter to reduce the federal role have been warmly endorsed and built upon by the new Administration.

Efforts to reduce the burden of health, safety and environmental regulations, for example, have already been expanded. As expected, Mr Reagan has proposed, along with his budget reductions, a set of regulatory reforms which would submit all new and existing regulation to strict cost-benefit analysis.

Similarly, additional patent reform legislation has already been introduced into the new session of Congress which would expand on Mr Carter's patent reform bill giving universities and small businesses patent rights on federally funded research.

David Dickson

UK research councils Allen accused

The UK Science Research Council is being hauled over the coals for sloppy bookkeeping. Its chairman, Sir Geoffrey Allen, is to appear before the Public Accounts Committee of the House of Commons on 17 March to explain irregularities in the council's funding in the financial year 1979-80. Details are hard to obtain because the council, usually frank, is saying nothing for fear of offending parliamentary privilege.

Part of the problem stems from the cash bonus that Mrs Shirley Williams, then Secretary of State for Education and Science, obtained for the research councils in 1978. The council's share was £33 million over the four financial years

1979-83. A circular was thereupon sent around universities asking for applications to the council to replace worn-out equipment: truck-loads of applications followed, worth £37 million, of which the council awarded £7.5 million. In the event, a change of government followed, funds were cut and the council received only £5 million.

Another question mark hangs over the university grants current in March 1980, which represented an increase in value of £31 million (34 per cent) over the previous year. There appears to be no indication that this large increase was planned. The exact amount of overspending remains unclear, but much of the money was spent by the Science Board (responsible for such topics as physics of solids and liquids, chemistry and biology). The new Spallation Neutron Source at the Rutherford-Appleton Laboratory at Chilton seems to have been a principal beneficiary.

Other misdemeanours are procedurally more serious. The Auditor General, Sir Douglas Henley, has already complained that the postponement of certain payments into the 1980-81 financial year, as part of an attempt to alleviate the financial deficit, contravenes government regulations. The Public Accounts Committee will also, no doubt, be asking about the council's calculation that the capital value of a site near Slough, yet to be vacated, could be regarded as a part of the income for 1979-80.

The council appears to have been the victim of government financial vagaries combined with inflexible accounting procedures. But the extent to which the problems are also self-generated will not be clear until 17 March.

Sir Geoffrey Allen, formerly the council's accounting officer, came to the end of his spell as chairman in October. The name of his successor is expected to be announced within a few weeks.

Philip Campbell

Soviet research

More home growth

The new Soviet Five-Year Plan calls for all branches of the economy to be brought up to the "most up-to-date levels of science and technology". Just how to do this is clearly causing the Soviet leadership considerable anxiety. At the Twenty-Sixth Congress of the Communist Party of the Soviet Union last week, Mr Brezhnev called on the whole scientific establishment to reassess the research and development basis of Soviet industry and to propose ways of regrouping the "scientific forces".

Not only the Academy of Sciences and the State Committee for Science and Technology should take part in this audit, said Mr Brezhnev, but also the science-based industries, including defence. Since Soviet military research is organized quite

separately from the civil sector, this last proposal suggests genuine concern, not simply congress window-dressing.

Mr Brezhnev singled out a number of fields of technology where "impermissible sluggishness" had led to delays in implementing "promising developments" — the continuous casting of steel, powder metallurgy, custom-built DC transmission lines and high-strength artificial fibres. Falling behind foreign competitors, he said, leads to massive expenditure of foreign currency for equipment and technology which the Soviet Union could have produced at home. Soviet potential technological self-sufficiency has been a feature of propaganda speeches since the January 1979 United States embargo. Mr Brezhnev's speech, however, referred rather to one of the major concerns of Soviet research policy: why is there often so long a gap between obtaining a new result and implementing it in production?

Mr Brezhnev suggested two possible lines of reorganization, which appear mutually contradictory. On the one hand, he stressed the Central Committee's support for an increased responsibility for the Soviet Academy of Sciences, and argued a "flexible and mobile" organization of research that would not tolerate "fruitless laboratories and institutes", but would respond "attentively" to the needs of scientists for equipment, instruments and pilot plant facilities. Taken in isolation, these remarks suggest more scope for serendipity and the capacity to switch rapidly from one line of research to a more promising alternative.

Mr Brezhnev went on the say, however, that the major sciences (including basic research) should concentrate more on solving "key national economic questions" and "discoveries capable of making genuinely revolutionary changes in production". The formulation of these tasks, he said, is the task of the central planning bodies and the State Committee for Science and Technology. The exact spheres of competence of the Academy and State Committee are frequently difficult to define, and Mr Brezhnev's speech does not make the issue easier. The previous Congress (1976) had made the Academy responsible for coordinating all science throughout the country, and although, to judge from the report to Congress of Dr Anatolii P. Aleksandrov, the Academy's president, much still remains to be done, there is no suggestion that the task should be taken out of the Academy's hands.

Dr Aleksandrov's report, moreover, reviewed a wide range of recent achievements, from particle physics and cosmology to the utilization of Estonian shales and the need to develop coal liquefaction and gasification techniques. Academy scientists, he said, have made notable advances in thermonuclear fusion, and in prolonging the life of agricultural machinery.

Discussion of future plans, at a Congress

of this type, is always to a large extent, orchestrated. A clear example was the intervention of Sharaf Rashidov, Party chief of the Uzbek SSR. Mr Brezhnev had called for a new food production programme. Mr Rashidov suggested that a "tremendous contribution" could be made by diverting the northern rivers to the Volga Basin and the Siberian rivers to the steppes of Kazakhstan and Central Asia. Plans for such proposals, however, are of course already well under way, and a considerable engineering effort earmarked for them in the new Five-Year Plan.

Dr Aleksandrov, however, had a lightertouch. One of his major proposals began as an apparent piece of by-play with Mr Brezhnev and the Leningrad Party chief Grigori Romanov, about the development of an anti-influenza spray which Mr Brezhnev had requested at the last Congress. (The very fact of such jesting is, in itself, a suggestion that the Academy's auspices are exceptionally favourable at present). The successes which Dr Aleksandrov reported — 10 million doses of the spray produced in 1980, 25 million scheduled for 1981, and the incidence of influenza in Leningrad cut by two-thirds, were simply a build-up. Soviet microbiological production, suggested Dr Aleksandrov, should be reorganized under a special Ministry of the Biotechnical Industry. **Vera Rich**

German nuclear power

Closures and delays

Four German nuclear power stations, representing a third of the country's 10,000 MW of nuclear power, are to close for at least a year for the complete replacement of their primary cooling circuits. The Bonn Nuclear Safety Commission ordered the closures after deliberating for three years over this issue, involving General Electric Company-designed boiling water reactors (BWRs) constructed under licence between 1973 and 1979.

Small leaks and cracks had developed in the primary circuit of these reactors which carry cooling water through the nuclear cores. The cracks appeared to be spreading by stress corrosion (combined effects of mechanical and thermal stress and chemical corrosion). The commission has concluded that uneven quality, poor choice of materials, and a thin gauge of steel for

the pipework were to blame, and that the circuits must be replaced to avoid the danger of a potentially catastrophic cooling failure. The cost of replacement has been estimated at DM 1,200 million (£250 million), with an equal sum for the loss of electricity sales. However, the four responsible utility companies have decided not to close down all the reactors simultaneously. One is already being refitted, and should be on-power again by the summer; and the others will be dealt with in sequence.

The boiling water reactors have not been a success, and Germany is now concentrating on the Westinghouse pressurized water reactor design, originally licensed by Siemens. Siemens and the constructors, AEG, are now merged in the company Kraftwerk-Union, and the excessive cost-trimming which may have led to the BWR problems is not expected again.

Meanwhile, the federal government's nuclear policy continues to be threatened by opposition from the left wing of the Social Democratic Party, particularly in Hamburg where construction of the Broksdorf reactor has been brought to a halt by demonstrations. Over the weekend, 50,000 people demonstrated at Broksdorf, and 127 policemen were injured. According to some observers, many of the demonstrators were not local, but represented national left-Social Democratic, Communist and Green Party groups. Broksdorf thus appears to have become a symbol of left-right conflict in Germany. **Robert Walgate**

Human growth hormone

Pituitary slump

Britain's supply of pituitary glands, from which human growth hormone (HGH) is extracted to treat 600 British children who do not produce it normally, has fallen by at least a third in the past year — to a level too low to keep up with demand. This has happened since responsibility for HGH extraction shifted, in June last year, from two Medical Research Council-funded researchers to the Department of Health and Social Security (DHSS). Bureaucracy has strangled their collection procedure, Dr Philip Lowry, one of the researchers, said last week. The collection rate has now stabilized at around 42,000 glands a year, DHSS claims, accounting for almost all patients who end up in a National Health Service mortuary — but the supply from public mortuaries (where victims of violence and accident are given postmortem examinations) has almost dried up. In the last year of the Medical Research Council scheme, 70,000 pituitaries were collected, said Dr Lowry.

Extraction of the hormone is now done at the Centre for Applied Microbiological Research at Porton Down, which has become increasingly involved in health

service functions since it was transferred to the Public Health Laboratory Service from the Ministry of Defence two years ago. Of two processes, depending on the form in which pituitaries are collected, Porton chose the older, using acetone-dried glands. This yields around 8 clinical units of HGH per gland as opposed to 15 by the newer frozen-gland system (which has been adopted in New Zealand and Canada), but collection is easier: the extracted gland needs only to be popped into a bottle of acetone, instead of a container packed around with dry ice. However, says Dr Lowry, who developed the frozen-gland process, clinical trials over the past year have shown that the HGH produced from frozen glands is the more pure, provoking no immune reactions — unlike acetone-dried HGH, which often contains modified and agglomerated versions of the protein, and can be rejected by the child.

The 336,000 units of HGH made available by the Porton process each year in Britain are enough to supply only 430 of the 600 children with a full dose of 15 units a week. So far, Porton has been relying on an excess of material collected through the old system; but soon the reduced supply of glands must have an effect on the supply of HGH. It may then be necessary to buy HGH from foreign producers, where market pressures have pushed up the price to 50 times the cost of home-produced hormone. Porton is also working on producing HGH from *Escherichia coli*, genetically engineered to excrete the hormone, provided by the Swiss firm Kabi-Vitrum in association with the California-based genetic engineering company, Genentech; but production is not likely to be significant for a year, at least until clinical trials, now under way at Great Ormond Street Children's Hospital on Genentech-produced hormone, are complete. Moreover, the genetically-engineered hormone is slightly modified: it has an extra amino acid (methionine) tacked on one end, and there is a possibility that, like agglomerated HGH, it may provoke an immune response.

Meanwhile, the DHSS is trying desperately to restore supplies of pituitaries from public mortuaries. But the department is facing numerous legal and personal obstacles. Legally the coroner must consent, and the relatives give written permission. But a death involving a public post mortem is usually an unexpected one, relatives are shocked, and the case does not seem so compelling as for a kidney or a heart. The DHSS recently sent 200 letters and made as many telephone calls to coroners and pathologists requesting their urgent cooperation — but the department has little hope that the supply will rise much in consequence.

A few months ago, supply problems were even more desperate, when hospitals too were failing to provide the glands. This may have been because morticians, in March 1980, negotiated a new contract

In addition to the four boiling water reactors (producing 3,300 MW), Germany has another six power reactors producing 6,600 MW. Another nine, to produce around 10,000 MW, are under construction, including the SNR fast breeder research reactor, and a high temperature reactor near Cologne. Construction is halted at two further sites (Broksdorf and Wyhl because of local and political opposition).

under which their wages were increased to cover extra duties — which included the extraction of pituitaries. Previously they had been paid on a "piece rate" — 20 pence a gland — so they now had no incentive for their unpleasant task. Pressure from hospital authorities on the workers, however, brought a response. Public mortuary workers also now receive nothing where they received 20 pence per gland before, so this may also be a factor in the HGH supply shortage. Negotiations are under way, says DHSS, on how these workers might be compensated by the area hospital authorities for providing the glands.

Some officials are suggesting that the real cause for the fall in supply is the "regularization" of collection procedures, compared with a little bit of horse-trading before. In the past, acetone sample bottles have been sent through the post; this is strictly illegal (acetone being inflammable) and has now been stopped. Also, extraction is now being done under full containment because of the danger of the propagation of slow virus from an infected gland (the pituitary being nervous tissue).

And were relatives always informed? Dr Lowry claims they were, and that he thus obtained more than half the pituitaries available from public mortuaries in London. The problems he lays squarely at the door of the DHSS. **Robert Walgate**

University of London Storm ahead

The reorganization of the University of London promises to be a more turbulent process than was foreseen a year ago. The plan that the Swinnerton-Dyer committee should make its final recommendations by the end of this year has been foreshortened by the mounting sense of urgency within the university. The committee has now agreed, with some misgivings, to produce its recommendations before the beginning of the next academic year. Meanwhile, the basis of the committee's interim report has been questioned by a document now circulating within the university, and likely to colour the arguments put by the Association of University Teachers to the committee at a meeting next week.

That document questions the arithmetic leading to the conclusion that the university's income will fall by between £15 and £20 million by the end of this decade. This is held to involve the unwarrantable assumption that the 3½ per cent cut in university finances announced last December will be permanent, the unnecessary assumption that overseas students attending London colleges will be charged only the minimum tuition fees decreed by the government and inconsistent assumptions about the basis used for calculating the loss of income if the numbers of overseas students decline. Briefly, the committee's calculation for the university as a whole is said to exclude the allowance made by the University Grants

Committee for part-time students.

This and other documents will form the basis on which the union will draw up a formal reply to the Swinnerton-Dyer committee's interim report, probably before the end of the month. Apart from arithmetical arguments, the union is concerned at the negative tone of the interim report, which is said to have neglected the university's advantages, in particular its attractions for overseas and part-time students and the relatively favourable age structure of its academic staff.

This view is likely to be echoed by the responses from individual colleges in the university, expected by Easter. Another view gaining ground is that the committee's interim report, with its comprehensive analysis of how the university spends its money, is sufficient, and that it should be for the university rather than the committee now to say what should be done.

The pitfalls for such committees are nicely illustrated by the troubles which have befallen the Flowers report on the organization of the medical schools in the University of London. After the rejection of the Flowers recommendations by the senate of the university last October, the

Soviet activists honoured

Three leading Soviet human rights activists have recently been honoured by Western scientific bodies.

The most prestigious award, foreign associateship of the French Academy of Sciences, was conferred on Dr Andrei Sakharov on 16 February. This, however, according to M. Paul Germain, one of the two secretaries of the academy, was in no way a political act, but was simply in recognition of the great importance of Sakharov's work in various fields of physics.

The Catholic University of Louvain in Belgium however, took a double view. When challenged by the Soviet embassy in Brussels that they should not award an honorary doctorate to Viktor Brailovskii, the Moscow refusenik cyberneticist, on the grounds that he was a "criminal and a prisoner" the rector replied that they were conferring the award in recognition of his contribution to the field of science and for maintaining the weekly seminars (for refusenik scientists).

Finally, the Royal College of Psychiatrists in London, in conferring an honorary fellowship on Dr Semeon Gluzman, did so specifically in connection with his work for human rights, for Dr Gluzman's chief contribution to psychiatry was the co-authorship (with Vladimir Bukovskii) of the *samizdat* "Handbook of Psychiatry for Dissidents" (a manual on how to resist psychological and pharmacological pressure), for which he is now serving a three-year sentence in Siberia following seven years in a labour camp.

Joint Planning Board set up a working party to make a more detailed analysis of the costs of medical education in the University of London. The report, based on an analysis by a firm of professional accountants, suggests that the cost savings obtainable by closing various clinical and preclinical schools are not those forecast by the Flowers committee, and that none of the options so far discussed would yield as large a saving on the total cost of £32.8 million as a modest change in the staff-student ratio.

Chemical and nuclear weapons Scientists speak out

Two groups are being set up to give British scientists a stronger voice on the development of weapons of mass destruction. On 26 February, the Russell Committee Against Chemical Weapons, a branch of the Bertrand Russell Peace Foundation, launched a campaign against what is seen as a chemical arms race. And at the end of this month, a conference is being held at the Open University, Milton Keynes, to inaugurate Scientists Against Nuclear Arms, a group committed to providing scientific information on nuclear weapons.

Scientists Against Nuclear Arms says that it has already received several hundred letters expressing interest and support. And 19 signatures were attached to the appeal for names of those opposed to chemical weapons when it was launched last week. One notable signatory, Dr Frederick Sanger, not distinguished as a signer of petitions, is particularly concerned with the possible use of new techniques in biology to develop more sophisticated chemical weapons and with the distortion of research priorities that could result from a chemical arms race. These issues, and uncertainties about the British government's future policy, have prompted the appeal, which asks scientists not to take part in research on chemical weapons and the British government not to stockpile them.

Despite repeated assurances that there are no plans to store or manufacture chemical weapons in Britain, the appeal's organizers fear that the British government's policy may change in response to pressure from the new Reagan Administration to house stocks of new binary chemical weapons on European soil. Last year, the United States announced that it was building a plant to manufacture binary weapons.

Believing the present to be a time of uncertainty, the appeal's organizers say there is an urgent need for public and parliamentary debate. Scientists have an important role, they argue, because the development of chemical weapons could be influenced more directly than that of any other type of weapon by research in non-classified laboratories. **Judy Redfearn**

CORRESPONDENCE

Data bank

SIR — The news item "Nucleotide sequences: Too many banks" (*Nature* 15 January, p.112) implied that we do not yet have a nucleic acid sequence data bank: in fact our data base system contains the largest collection of nucleotide sequences in the world, updated on a regular basis and accessed through a sophisticated computer retrieval system.

The data bank was first made available to the scientific community by telephone link to our computer on 15 September 1980, when the information base contained over 227,000 residues of DNA and RNA sequences published before 1 August 1980. During a three month demonstration project, the collection was updated monthly with material published mainly between August and October. The final total on 12 December 1980 was 350,000 residues.

We are continuing to update the data base and to make it available on a subscription basis. We are now processing more than 40,000 residues a month, and a book is being prepared which will include the information in the collection and describe the retrieval system and its uses.

The data bank is supported by the Institute for General Medical Science of the US National Institutes of Health, the National Aeronautics and Space Administration, and contributions and subscriptions from foundations and industrial concerns. The expense involved in maintaining a complete, critically reviewed and up-to-date data base is too great for it to be operated on a voluntary basis, so other means of support must be sought. Our data base is available on a subscription basis (for the use of the subscriber in his own institution, not for redistribution). The collection will be made available on this basis to the European Molecular Biology Organization for use at Heidelberg, under the direction of Dr Greg Hamm, so that the interests of the European non-commercial research community can be considered.

The cost of such a data base is usually justified by the resulting benefit to a research area. If all those organizations that could benefit from a nucleotide sequence data base were to contribute some support, there would be sufficient money to establish a continuing project.

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Antipodean 2,4,5-T

SIR — There is continuing concern in Australia regarding the possible deleterious effects of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on animal life, especially human beings. The question of a link between 2,4,5-T usage and congenital abnormalities has been raised on several occasions¹⁻³. Concerns of this nature were major reasons for withdrawing 2,4,5-T-based defoliant (such as Agent Orange) from use in the Vietnam war during the early 1970s. Many batches of defoliant

contained high quantities of the extremely toxic contaminant 2,3,7,8-tetrachloro-cyclodibenzo-*p*-dioxin, suspected of being a teratogen. International sale of the unused defoliant was apparently considered by the US government⁴ and some of this defoliant may have been imported into Australia⁵.

On 18 August 1971 the Australian government initiated enquiries under the provisions of the Customs Tariff (Dumping and Subsidies) Act regarding the importation of "salts of 2,4,5-trichlorophenol". Two years later, dumping duties were imposed on goods based on the esters and salts of 2,4,5-T, *retrospective* to 4 January 1971. It seems likely that large quantities of such goods were bought very cheaply overseas and sold on the Australian market in the late 1960s or early 1970s.

The chemicals 2,4,5-T and 2,4-dichlorophenoxyacetic acid which make up the defoliant Agent Orange would normally be classified as pesticides in trade statistics. However, they could be given a chemical classification and assigned the 1972-3 SITC code number 512.28.09 ("other phenol derivatives, halogenated etc."). In the financial years 1969-70 and 1970-71 over 300 tonnes of chemicals with this SITC code are recorded in Australian trade statistics as imports from Singapore (the "country of production"). None of this material is listed as an export or re-export of Singapore in that country's *External Trade Statistics*, and there have been no subsequent imports from Singapore. (A small import from Singapore is recorded in 1968-69, but there is nothing before this.) The Australian Bureau of Customs has declined to be of any assistance in determining the true nature or origins of these goods.

With the help of the Australian Bureau of Statistics we have ascertained that in 1970-71, 290,000 lb of these imports entered Australia through Queensland, and another 22,400 lb through Western Australia. The *Australian Chemicals Guide (Industry Study)* shows a very limited number of companies involved in the processing of halogenated phenols. With the assistance of the *Guide* and the reference *Who owns Whom* it is possible to obtain a very short list of potential importers.

The Australian government has access to the records which would permit a thorough investigation of this matter. The results of such an investigation could have a vital influence on a multi-million dollar epidemiological study at present being undertaken by the Australian government on the effects of defoliants on Vietnam veterans. The study envisages a comparison of veterans exposed to defoliants with those "unexposed" veterans who did not serve in Vietnam.

Further details of this matter appear in a forthcoming paper⁶.

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Cell contamination

SIR — The editorial, "Responsibility for trust in research" (*Nature* 22 January 1981, p.211) was excellent. Our group sends various human cell lines to laboratories all over the world. We caution scientists about the dangers of cell-cell contamination and if we read an article with strange conclusions that involved one of our many hundreds of cell lines, we offer to re-authenticate its identity without cost. Often the cell line in question has been passed from one laboratory to another. We have been discouraged to detect publications in which the scientists have continued to use a contaminated cell line even after the error has been pointed out to them. Biochemists in particular seem loath to seek biological advice. One chemist replied that the kind of cells that he used wasn't really important.

Actually there are other serious contaminations which affect research with cells. Most cell lines are cultured in media supplemented with antibiotics and often the cultures are contaminated with slow-growing bacteria and mycoplasma. It is difficult to detect minimal numbers of these organisms. Electron microscopy of a pellet of centrifuged media may reveal them.

We do not agree that the responsibility for monitoring the validity of research rests with the department head. Departments are too complex and even the division heads may not have the competence to evaluate a specific "bench" operation. The scientist himself and his associates in a "specialty" must exert the self discipline of avoiding, acknowledging and correcting errors.

As the use of enzyme profiles and new techniques of cytogenetics and the detection of "specific" antigens and cell products evolve, it will be easier to detect cell-cell contamination — perhaps even hybridization. Our methods for authenticating cells are still relatively primitive. The establishment of authorities to referee cell-cell contamination is premature. The donor and recipient of a cell line share the responsibility of the authenticity of the cell line.

Identification of a cell line should include: (1) data about the original collection of tissue and pathological diagnosis, (2) serial numerical records of all subcultures, (3) electron microscopy — preferably of both the tissue of origin and the cultured cells, using special stains if critical, (4) isoenzyme profiles, (5) karyotype analysis of chromosome morphology after banding, (6) cell products, (7) studies of growth in immunosuppressed animals such as nude mice, and (8) growth characteristics in various media.

The errors associated with cell lines have probably had an infinitesimal effect on the fabric of scientific progress in comparison with other ridiculous and shameful pronouncements. For example, governmental carcinogens, the clinical effectiveness of interferon, and numberless epidemiological studies such as telephone surveys of complex disease states.

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NEWS AND VIEWS

Carbon dioxide and climate: ice and ocean

from Starley L. Thompson and Stephen H. Schneider

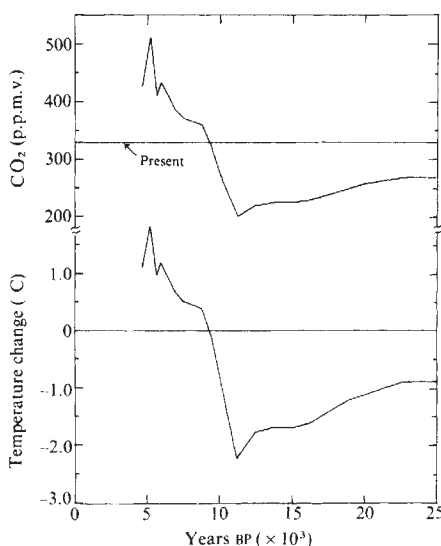
THE presence of carbon dioxide in the atmosphere has long been known to affect climate through the so-called 'greenhouse' effect. The present rate of growth of atmospheric CO₂ concentration due to fossil fuel burning will result in a doubling of the CO₂ level sometime in the next century and this may produce a global surface temperature increase of between 1.5 and 4°C. Since this temperature change is as large or larger than any natural change in the past few millenia, the increasing interest in the connection between CO₂ and climate is not surprising. Recently, new ground has been broken in two relatively unexplored areas — the measurement of natural CO₂ variations on long time scales and the role of the ocean in modulating climatic response to the anthropogenic CO₂ increase.

The absence of firm evidence of past CO₂ variations has been a troublesome gap in our knowledge. A correlation between levels of CO₂ and palaeoclimatic temperature data would constitute the first clear empirical evidence of CO₂-induced climatic change.

Two recent papers have shown that past levels of atmospheric CO₂ concentration can be reconstructed from the CO₂ trapped in large ice sheets. Berner *et al.*¹ and Delmas *et al.*² have analysed trapped air in polar ice cores and concluded that large variations of atmospheric CO₂ occurred during the past 30,000 years. Previous attempts to analyse the total CO₂ content of the ice cores failed, but new extraction techniques increase the researchers confidence that the measured CO₂ variations may reflect accurately the CO₂ variations in the atmosphere.

Two features stand out in the accompanying figure from Berner *et al.*¹. First, their data show a CO₂ concentration of as little as 200 p.p.m. by volume 10–30 years ago compared with a present-day value of around 340 p.p.m. Second, there appears to have been more atmospheric CO₂ in the mid-Holocene (about 5,000 years ago) than at present. Estimates from

current climatic models of the global surface air temperature change caused by such CO₂ variations are also shown. Uncertainties in present climatic theory suggest that these temperature estimates should be within a factor of two of the actual CO₂-induced temperature changes — assuming, of course, that these preliminary measures stand up to further analysis. Note that the estimated global temperature changes range from +1.8°C to –2.2°C. These represent a large fraction of the temperature range deduced from other palaeoclimatic evidence for the period.



Graph showing the relationship between the atmospheric CO₂ level reconstructed from Camp Century, North Greenland, core measurements (upper curve) and the corresponding global change in surface air temperature estimated from current climatic models (lower curve). (Taken from ref. 1.)

Should these large CO₂ variations prove to be reasonably accurate, the implications for palaeoclimatic theory would be enormous. One of the most difficult problems in palaeoclimatic modelling has been the determination of the feedback processes which must exist to cause the large-amplitude glacial to interglacial transitions³. A feedback including natural CO₂ variations like those in the figure could explain as much as half the surface temperature amplitude of the ice age/inter-

glacial climate changes. However, it does not appear that the CO₂ variations initiate these large climate changes as the minimum and maximum CO₂ concentrations do not appear to lead the respective glacial and interglacial extremes of 18,000 and 6–8,000 years ago. Therefore, CO₂ changes may act only to amplify climate changes that are initiated by other means.

In addition to improving our understanding of past climates, any evidence that CO₂ levels were higher during the mid-Holocene than today may give more credence to the idea of using this climatic 'optimum' period — when surface temperatures were a few degrees warmer than today — as an analogue for the climatic change which may be induced by a future anthropogenic CO₂ increase⁴.

A new area of research on the CO₂ problem has been recently explored by Hoffert *et al.*⁵. Previously, most climatic modelling studies examining the effect of increased CO₂ have considered the models' response in a state of climatic equilibrium. For example, a researcher would perturb a mathematical model of climate with a fixed, time-independent doubling of the CO₂ concentration and allow the model climate to reach a new equilibrium. This is a reasonable approach to take when testing the various models since it affords an order-of-magnitude estimate of climatic response for a fixed forcing (for example, when the CO₂ concentration has doubled), while meeting the constraints of a limited computing budget. However, the actual CO₂ increase is a continuous rise, not an instantaneous step increase. In addition, because of its large heat capacity, the ocean acts as a thermal buffer by delaying the response of the atmospheric temperature — an effect that has been neglected or undervalued in most equilibrium studies.

In order to simulate adequately the climatic response to the actual time-dependent CO₂ increase one must account for oceanic heat capacity, which means that one must determine how the ocean mixes the excess CO₂ heating at its surface. The upper layer of the ocean is mechanically well mixed by surface winds to a depth of approximately 50 metres. Including the heat capacity of the mixed layer alone would delay the global temperature response to the CO₂ increase

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by 5 to 10 years^{6,7}. But the mixed layer is only a small fraction of the ocean volume. Mixing occurs throughout the depth of the ocean, although at much slower rates than within the mixed layer. When Hoffert *et al.* simulated the heat capacity of the entire ocean in an ocean model with vertical diffusion and specified upwelling, they found that the response of the global temperature to the CO₂ increase would be delayed by 10 to 20 years by the year 2,000. Even longer lags would occur in the next century.

The effect of such a global time lag will have to be considered when examining

future climatic data for the predicted CO₂ warming. We may not be able to extract a detectable CO₂ 'signal' from the climatic 'noise' (ref. 8) until several decades after the date estimated from equilibrium calculations. Thus the absence of unambiguous warming during the remainder of this century would not serve to disprove the validity of our current *equilibrium* estimates of the effect of CO₂ on global temperature.

However, the determination of the global temperature time lag cannot be divorced from the complex problem of modelling the regional variations in oceanic mixing. As much of the communication between the deep ocean and surface waters occurs in downwelling polar seas, Hoffert *et al.* concluded that the as yet poorly understood response of the polar oceans to the CO₂ increase may be very important in determining the global time lag.

A related idea (discussed by Schneider and Thompson⁹) is that variations in oceanic mixing which create a geographical distribution of effective oceanic heat capacity may also serve to produce thermal time lags with substantial regional

variations. The significance of this goes beyond the added difficulty in unambiguous early detection of the global CO₂ warming. Different thermal lags for different regions implies that anomalies in surface temperature gradients, and any associated regional climatic anomalies, may with time become substantially different from those computed in an equilibrium simulation. Since it is the potential regional climate changes which are of the most economic concern, we feel that improving our understanding of the nature of the transient climatic response should be a major priority for future CO₂-climate research. This means more coupled atmosphere-ocean-cryosphere modelling studies.

Finally, we should point out that the problem of the transient climatic response is just one of a set of interdisciplinary problems. How much fossil fuel will be burned, the global carbon cycle, the global and regional equilibrium climatic response, the transient response, the physical and societal impacts of climatic changes, and possible policy responses to prevent or adapt to CO₂ effects are all important aspects of the 'CO₂ problem'. □

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Active galaxies: dragon hunting in Arizona

from Martin Elvis and Andrew S. Wilson

PLOTS of the electromagnetic spectrum of active galaxies covering the ten decades of frequency from radio waves to X rays were described at a recent workshop* and show just how rapidly this field is advancing. The successes of the radio astronomers' Very Large Array (VLA) and the HEAO X-ray observatories enabled radio and X-ray astronomers to meet their optical colleagues on nearly equal terms for the first time. The sensitivity and angular resolution of the new instruments is bringing some order into the classification of active galaxies — the old diversity now appears to have been largely the result of observations limited to only the extreme examples of particular characteristics.

The meeting was restricted to active galaxies and conspicuously avoided their close relatives, quasars. As a result there was an emphasis on objects in which resolved structure could be seen. The most striking examples of this came from the detection of over 50 jets by the VLA (reviewed by Bridle, VLA, New Mexico). These long, collimated structures point from the nuclei of bright radio galaxies to their giant radio lobes. They needed a dynamic range approaching 1,000:1 to be discovered close to the bright nuclear sources but, now that this has been achieved, observations of such jets have

become common, almost ubiquitous. Over 60 per cent of the nearby ($z < 0.05$) 3CR radio galaxies have them. The jets are seen on all size scales from 1 parsec to 1 Megaparsec. Often they are one-sided, perhaps as a result of relativistic beaming. In all likelihood we are seeing the beams of relativistic particles which Rees proposed ten years ago would power the giant radio lobes. Polarization measurements show that the magnetic field is often parallel to the jet near the nucleus but turns at right angles to the jet further out (although sometimes leaving a parallel region at the edge). There is a suggestion that the parallel region is longer for higher radio core luminosities. If such a correlation is borne out it will provide useful information for the beam instability models discussed by Blandford (Caltech), Laing (NRAO, Virginia) and van Bruegel (Leiden).

It is necessary that the jets are confined in some way. The simplest possibility is that the galaxy and the jet are surrounded by hot gas. This should be visible by X-ray emission and observations have been made from jets in the three classic sources: M87, Cen A and 3C273 (Schreier, Harvard-Smithsonian Center for Astrophysics). However, the detailed correspondence of the X-ray, optical, and radio features suggests strongly that the X rays are a continuation of the jets synchrotron spectrum. For other 3CR radio galaxies initial results of the *Einstein* survey

(Fabbiano, Harvard-Smithsonian Center for Astrophysics) require equipartition fields within the jets of the order 10^6 – 10^7 gauss. If these radio jets are thermally confined the expected X-ray emission from the confining gas is just below the current detection threshold.

Miniature double sources have been found by Wilson (University of Maryland) in five Seyfert 1 galaxies. Type 1 Seyferts are the archetypal 'radio-quiet' active galaxies so this discovery is particularly interesting. It demonstrates that the same linear symmetry we see so clearly in the radio-loud case applies to this other, much more numerous, class. Since these Seyfert 1 galaxies have clear spiral structures it is simple to see whether the direction of radio symmetry aligns with the rotation axis. Such convenient alignment does not appear to occur.

Before leaving jets the extragalactic astronomers were presented with a 'working scale model' of their problem. Hjellming (VLA, New Mexico) presented a beautiful series of VLA observations of SS433 showing a complex series of changes with time. He was able to explain these with a simple, constant velocity, ballistic blob model. Whilst this is surely too simple for the extragalactic case it must have some

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*A workshop on active galaxies was held in Tucson, 19–23 January 1981 and was sponsored by the Kitt Peak National Observatory.

suggestive power for those trying to understand the larger scale phenomenon.

The other three days of the meeting were used to examine and compare observations of the compact nuclei themselves. Weedman (Pennsylvania State University) presented a useful criterion for distinguishing the emission line galaxies that need a non-thermal ionizing continuum from those that can be explained solely by bursts of star formation. He found that for emission line widths greater than 250 km s^{-1} at least 90 per cent of galaxies needed, as he said, 'a Dragon in the middle' — a general term for any source of a non-thermal continuum. For narrower line widths no dragon is needed.

Far from being discarded for their lack of activity narrow line 'starburst' nuclei emerged as a new area of study with radio (Gisler, NRAO, Virginia), $H\alpha$ emission line (Heckman, NRAO, Virginia) and X-ray (Fabbiano, Harvard-Smithsonian Center for Astrophysics) images providing new inputs. Why some galaxies undergo intense star formation periods is as little understood as why other galaxies have non-thermal active nuclei.

Further clarification of what constitutes an active nucleus came from comparing the X-ray (Mushotzky, Goddard Space Flight Center) and optical (Huchra, Harvard-Smithsonian Center for Astrophysics) luminosity functions for active galaxies.

These now agree (to a factor two) so the two selection techniques — hard X-ray emission and broad optical emission lines — do find the same population of objects.

The 'narrow emission line galaxies', once thought to be a different class, have the universal power law X-ray spectrum of slope 0.7 above 4 keV that all measured Seyfert 1 galaxies possess (Mushotzky). Also, better optical spectra showed faint broad wings on their Balmer lines, just like Seyfert 1 galaxies.

Some anomalies remain: No-one is sure whether or not Type 2 Seyfert 1 galaxies (differing from Seyfert 1 galaxies by the absence of broad hydrogen emission lines) have dragons in them. Also new Einstein identifications of active galaxy sources (Elvis, Harvard-Smithsonian Center for Astrophysics), include two that would not have been picked out by an optical survey since they have neither strong emission lines nor blue colours.

Much detailed theoretical work was presented, largely based on the concept of an accretion disk around a supermassive black hole. Although progress is being made in investigating these systems as demonstrated by, for example, Begelman (Berkeley), Duncan (Cornell) and Eilek (Institute of Mining and Technology, New Mexico), the models need to be explored further before the tenuous links between theory and observation can be strengthened. □

the presence of SV40 T antigen and that it occurs irrespective of whether the SV40 sequences are physically linked to the foreign DNA or merely co-injected with it.

The chief reasons for using viral vectors are to place foreign DNA sequences under the control of known viral regulatory elements, and to attach them to viral origins of replication so that the ectopic sequences can be amplified to high copy number in infected cells. Viral vectors have the further advantage that the foreign DNA can be delivered virtually synchronously to large populations of cells. Until recently, only one type of viral genome — that of SV40 — was used for this type of work. Typically, the late region of the viral DNA is replaced in whole or in part by foreign DNA sequences. Unlike bacteriophage λ vectors where the recombinant DNA molecules are packed *in vitro* into virus particles, the SV40-chimaeric DNAs are first mixed with a helper viral DNA and the mixture then used to infect permissive cells. Although only a small proportion of the cells can be infected with DNA, the susceptible cells efficiently take in both the recombinant DNA and its helper. The recombinant genome replicates and as long as it is not too large, becomes packed into viral particles. The resulting mixed virus stock can be used to infect enough cells to allow analysis of the expression of the foreign DNA by conventional biochemical techniques. Quite a large number of genes has now been propagated and expressed in this way. The most recent additions to the list are human growth hormone, hepatitis surface antigen, human genomic globin sequences (Pavakis, NIH) and the putative transforming protein, p21, coded by Harvey sarcoma virus (Khoury, NIH). In many cases the expression of the exogenous genes is surprisingly efficient, even when the foreign DNA is inserted into unpromising positions in the SV40 genome. For example, when a form of the rat preproinsulin gene from which all splice signals have been deleted is inserted into an SV40 vector which also lacks splice signals, recombinant mRNA is synthesized in large quantities under the control of the SV40 late promoter and translated efficiently (Gruss, NIH; see *Proc. natn. Acad. Sci. U.S.A.* 133; 1981). This result indicates that lack of splicing may not be as strong a deterrent as had previously been thought to the expression of ectopic DNAs.

Promising though the SV40 system is, it has suffered from two disadvantages. First, virus populations which contain recombinant genomes are always mixed, and sometimes the particles which carry foreign DNA sequences form only a small proportion of the virus stock. Second, the size of the foreign insertion is limited by the amount of DNA which can be packed into SV40 particles. However, several new vectors are being developed which promise to alleviate these difficulties. Bovine papilloma virus is capable of transforming

Designer genes

from a correspondent

SMALL MEETINGS on molecular aspects of biology are rare luxuries these days. Almost as fast as they well up, new ideas and discoveries become part of the mainstream, where they sustain shoals of workers most of whom are darting in the same direction at the same time. Consequently, it was refreshing to attend a small meeting* on 'Mammalian Vectors' during which the forty or so participants discussed work in varying stages of accomplishment which dealt, in surprising variety, with the problems of obtaining expression of ectopic genes in mammalian cells.

There are three methods in common use by which foreign genes (generally in the form of cloned fragments of genomic DNA or cDNA copies of mRNA) can be reintroduced into cultured mammalian cells: by microinjection; linked to segments of viral DNA; and by simultaneous transformation with two separate pieces of DNA — one carrying the gene of interest, the other a genetic marker which confers a

selective advantage on the recipient cells.

Each method has its own set of advantages and disadvantages and the three techniques therefore should be regarded as complementary rather than exclusive. Microinjection is an efficient way of introducing known quantities of DNA into cells whose exact location on the culture dish is known. Enough cells can be injected to allow detection by immunofluorescence of the products of expression of the injected DNA. Normally such expression is transient, lasting, at the most, for a few days. However, about one to two per cent of the injected cells can be developed into lines which express the foreign genes constitutively (Capecchi, University of Utah; Lo, University of Pennsylvania; French Anderson, NIH). Furthermore, Capecchi showed that the efficiency with which constitutive cell lines are established can be increased some 20-fold, by injecting together with the foreign gene, a segment of DNA which spans the origin of simian virus 40 (SV40) DNA replication. The cause of this improvement is unknown; it is especially puzzling that the effect does not depend on

*Held from December 11 to 13 at the Banbury Conference Center of Cold Spring Harbor Laboratory.

mouse cells to yield cell lines which, by contrast to similar cells transformed by SV40, contain 10–120 copies of circular viral DNA with no evidence of integrated viral sequences. Howley (NIH) has attached the rat preproinsulin gene to the transforming region of bovine papilloma virus DNA and has established transformed cell lines which contain episomal copies of the recombinant DNA. The cells contain spliced preproinsulin mRNA and secrete significant quantities of proinsulin.

Gluzman (Cold Spring Harbor Laboratory) has developed lines of permissive simian cells which constitutively express functional SV40 T antigen. They can therefore be used in the absence of helper to propagate recombinant SV40 molecules in which the early region of the viral genome has been replaced by foreign DNA sequences, or to amplify DNA molecules which carry the SV40 origin. Using this system, Mellon (California Institute of Technology) has attached to the SV40 origin cloned copies of human chromosomal globin genes. Forty-eight hours after the introduction of these chimaeric molecules to COS cells, the globin genes direct the synthesis of approximately 10^4 molecules of globin mRNA per cell. The mRNAs are spliced, polyadenylated and have 3' and 5' ends identical to those of normal globin RNA. These results show that cloned copies of chromosomal genes which carry their own control sequences and which normally are active only in specialized cells can be efficiently expressed when reintroduced to undifferentiated tissue culture cells and amplified to high copy number as part of an SV40 recombinant genome.

Two groups (Thummell, University of California, Berkeley; Solnick, Cold Spring Harbor Laboratory) have used defective adenovirus 2 genomes to propagate and express foreign DNA. Using different techniques, both groups have constructed a series of recombinants in which the expression of the early region of SV40 is placed under the control of the major adenovirus late promoter. Many of the recombinants synthesize large quantities of authentic SV40 large T antigen. This result opens the way to use of adenovirus as a vehicle for the propagation and expression of moderate-sized eukaryotic genes, as, at least in theory, adenoviral vectors can be constructed which are able to accept segments of foreign DNA 30–35 kilobases in length.

Finally, although other selective markers are being developed (methotrexate resistance, neomycin resistance, dependence on guanine phosphotransferase; Southern and Subramanian, Stanford University), thymidine kinase, cloned either from herpes simplex virus or from chicken DNA remains the marker of choice in co-transformation experiments. Using this technique, there is no difficulty in obtaining cell lines which contain a few copies of the foreign DNA of interest, in

the form of a large catenate formed by recombination between the incoming thymidine kinase gene, the carrier DNA and the foreign sequences themselves (Wigler, Cold Spring Harbor Laboratory; Ruddle, Yale). In general the level of expression of the foreign sequences is too low to be useful. Nevertheless, the system can be manipulated in interesting ways. For example, Kurtz (Cold Spring Harbor Laboratory) reported that the α -2 μ globulin genes which code for the major

urinary protein of rats can be introduced by co-transformation into cultured mouse cells. Several cell lines were obtained in which these foreign genes could be turned on by dexamethazone. The finding that co-transformed genes can continue to respond to hormones opens up the exciting possibility of analysing hormonally controlled genes by the same techniques that have been so successful in dissecting the fine structure of other viral and cellular promoters. □

Astrophysics and particle physics come together

from Frank E. Close

THE advances made in high-energy physics and astrophysics have enabled the 1970s to be described as the greatest ever in fundamental research. The discoveries of the J/ψ particle in 1974 and of charm in 1976 provided major evidence supporting the theory that united the electromagnetic and weak forces. Some of these particles' properties caused attention to be focused on a candidate theory of quark interaction, named 'quantum chromodynamics' or simply QCD. (The so-called 'strong' nuclear force is now viewed as a complicated manifestation of this more fundamental QCD force which acts on the quarks — the constituents of the subnuclear particles.) Although still some way from being verified, accumulating data do appear to fit in with theoretical expectations based on QCD.

The mathematical similarities among the theories of the electromagnetic, weak and 'strong' forces are so striking that theorists have tried to build a grand unifying theory which would include all the natural forces except gravity. The simplest way of implementing the theory has already successfully predicted the existence and mass of the fifth variety of quark (the so-called 'bottom' quark). Much experimental effort is now centred on the prediction that protons are unstable.

These topics were all reviewed at the annual meeting of the UK High Energy Theoretical Physicists held in December at the Rutherford and Appleton Laboratories.

J. Badier (Paris) reviewed experimental data on the behaviour of quarks inside protons and pions and together with R. Roberts (Rutherford Laboratory and CERN) discussed how well these and other data fit with the QCD theory of quark interactions. The answers continue to be very encouraging. Everything seems to fit with the theory, and although far from proved one increasingly feels that it would

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now require something totally unexpected to overthrow it.

Although the basic tenets of QCD are rather simple, the rich structure arising from them is still only poorly understood. M. Creutz (Brookhaven, New York) described some computer-based studies of QCD performed on a 'lattice' (where space-time is treated as discrete rather than as a continuum) which suggest that QCD leads to confinement of gluons (the QCD quanta, analogues of photons in QED) and thus, it is speculated, of quarks. Direct proof of this is still, however, unavailable. Some technical problems on the behaviour of QCD in the IR region were discussed by J. C. Taylor (Cambridge). Some feel that deeper understanding of this domain might resolve the confinement question.

Looking to future developments, searches for proton decays and their significance for grand unified theories of the natural forces were discussed. D. Perkins (Oxford) described the experiments planned or in operation. Present limits show that the proton lifetime is greater than some 10^{29} years. Perkins pointed out that logistic problems with experiments deep underground and with backgrounds from neutrinos would probably prevent this limit ever being pushed beyond 10^{33} years. Grand unified theories predict 10^{31+2} years — satisfyingly in this window! Observation of such proton decays in the near future could provide a major impetus for belief in grand unified theories.

The theoretical issues involved in constructing grand unified theories of the particles and forces were detailed by J. Ellis (CERN). In our cold world, energy densities are rarely much greater than 1 GeV per fm³ and the forces appear quite different from one another in their strengths and properties (see Dombey *Nature* **288**, 643, 1980). The unification of the forces is manifested at energy densities some 10^{15} times greater than these (note

that gravity is not important until densities of yet a further 10^4 are met, and so gravity can still be ignored). High-energy accelerators of subatomic particles can create locally energetic conditions and do appear to show a glimpse of the trends required if QCD is to be unified with the electroweak force, but we are still many orders of magnitude from 10^{15} GeV. However, such energy densities were probably common in the early Universe and it is here that a new merging of interests in high energy and astrophysics is taking place. These were described by Ellis and R. Taylor (Sussex).

Proton stability is particularly significant as several years ago Andrei Sakharov (Gorki) had noted that three ingredients were required if we were to understand the apparent excess of matter over antimatter in the Universe at large. One is an absolute distinction between them: this is indeed provided in nature by the phenomenon of CP violation (whose discovery led to the 1980 Nobel prize for Cronin and Fitch of the US). The second is an absence of thermal equilibrium: provided in modern cosmology theory by the big bang expansion. Finally, proton instability is required. This is indeed provided by the grand unified theories; we wait to see if it is also provided by nature!

Within the grand unified theories it has been argued that mechanisms naturally exist that will give rise to the observed quantitative matter excess. Ellis pointed out that the validity of this 'explanation' can be tested because the mechanism also generates an electric dipole moment for the neutron. At present there is an experimental upper limit that places this moment at less than about 10^{-24} e cm. Present experiments hope to lower this by three orders of magnitude. Ellis noted that the above theories provide a 'cosmological lower bound' some four orders of magnitude below the present limit! This should provide a significant spur to experiment and provides another example of how studies of subnuclear particles can reveal the mechanisms responsible for the development of the early Universe.

One of the more recent lines of investigation is into the question of whether there are new phenomena between the present energies and 10^{15} GeV (apart from the predicted production of massive W, Z particles of the electroweak theory and the postulated sixth quark and Higgs particles). In particular, the nature of the Higgs particles is unclear.

During the past year there has been much interest in the possibility that the Higgs particles are not elementary but are dynamically bound composite systems of more fundamental entities. E. Eichten (Harvard) described so-called 'hypercolour' or 'technicolour' theories that contain the Higgs particles as composite bound states and in some formulations require substructure to the leptons and quarks. These theories predict that a new ultra-

high-energy scale of phenomena exists in nature. Eichten described possibilities of testing these ideas at present energies. Experimentalists are urged to search for the light pseudo-Goldstone bosons predicted in the theory (possibly of a mass of about 10–20 GeV and producible in electron-positron annihilations) or to search for the rare decay modes that technicolour's existence can induce from the conventional particles (like $\mu \rightarrow e\gamma$, $K \rightarrow \mu e$).

Finally, in all these brave schemes to unify our understanding we come up against the perennial problem — gravity. How gravity fits into nature's scheme is still

something of a mystery. P. van Nieuwenhuizen (Stony Brook, US), one of the founders of 'supergravity' theory, described developments in this exciting field. Some of the technical problems that arise attempts to build a satisfactory quantum theory of gravity appear to be overcome if gravity is but part of a larger theory — supergravity. This theory is very elegant mathematically and yet, frustratingly, we have little or no indication that Nature has made use of it. van Nieuwenhuizen succinctly summarized the beauty of the mathematical structures by saying "I hope that Nature is aware of our efforts". □

Υ physics—from bottom to top

from Roger Cashmore

THE Υ meson was first observed in proton-proton collisions late in 1977 at Fermi National Accelerator Laboratory (FNAL). The meson had a mass of ~ 9.45 GeV and was seen in its decays to $\mu^+\mu^-$ and e^+e^- with a width consistent with the measurement resolution. (The width of a resonant state is related to its lifetime. Very short lifetimes lead to large widths. However, even a narrow state can appear to be broad if the experiment is limited by the measurement accuracy. The width then reflects the experimental resolution.) A further state, the Υ' , was also indicated at a mass of ~ 10.00 GeV.

These observations were quickly confirmed in experiments at the e^+e^- storage ring, DORIS, at DESY in Germany. The Υ and Υ' were observed as separate states, ~ 580 MeV different in mass, and since then the Υ has been shown to have a total width of 20–50 keV, a remarkable fact for such a high-mass state and rather reminiscent of the ψ . Generally, states of such high mass decay quickly into many lower-mass particles leading to large intrinsic widths of hundreds of MeV. Combined with the FNAL data, these accurate measurements of the masses of the Υ and Υ' states suggested the existence of yet a third Υ resonance at a mass just beyond the reach of the DORIS storage ring.

It appeared that the bound state system of a new quark, the charge $\frac{1}{3}$ bottom quark (b), and its antiparticle ($\bar{b}$) was being observed. The Υ was the ground-state $b\bar{b}$ meson and the Υ' its first excited state. New quarks, bottom (charge $\frac{1}{3}$) and top (charge $\frac{2}{3}$), had been implied by the earlier observation of a new lepton, the τ lepton, at the electron-positron storage rings SPEAR and DORIS, capable of reaching 7 and 10 GeV energies respectively.

The past year has seen the successful operation of a new electron-positron storage ring, CESR, at Cornell which can be used for experiments in the 8–16 GeV mass region. This covers the Υ mass range and allows much more detailed studies of this new quark system. The Υ

and Υ' states were confirmed and the third state, the Υ'' , observed at a mass of ~ 10.32 GeV. All three states had widths consistent with the machine resolution, suggesting that they were all narrow resonances.

However, as the beam energies were raised a fourth structure, the Υ''' , was observed at a mass of ~ 10.54 GeV but this had a width of ~ 10 MeV, larger than the machine resolution and in marked contrast to the other states. This suggests that the state is above the threshold for decay into $B\bar{B}$, B being a $b\bar{q}$ meson (where q is one of the more familiar u, d, s quarks) carrying the flavour of the new b quark. Thus these Υ states provide a laboratory for the study of both the bound systems of this quark, $b\bar{b}$, and the open flavour states $b\bar{q}$.

These two areas are vital in validating the present descriptions of the forces in particle physics. The strong force, which binds the quarks and antiquarks to form the mesons, is hypothesized to be indifferent to the quark flavour and only related to their colour through the exchange of coloured gluons between them. (Earlier results had indicated the need for three colours of each flavour of quark.) Hence $q\bar{q}$ systems should be similarly independent of the quark flavour. This model for the strong interaction is known as QCD (quantum chromodynamics). On the other hand, the weak force, now believed to be unified with the electromagnetic interaction according to the gauge theory of Weinberg and Salam, implies specific transitions between the different flavours of quark, for example, $c \rightarrow s$, $s \rightarrow u$. These hypotheses need quantitative verification.

In the $b\bar{b}$ bound system the mass difference of the Υ and Υ' is close to that of the ψ and ψ' , indicating that, although the new quark has a mass ~ 5 GeV in contrast to the charm quark mass of ~ 1.5 GeV, the quark forces (or potentials) are essentially

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unaltered. This is the remarkable result, that the force is flavour independent, which is expected if the strong forces are indeed due to the exchange of coloured gluons as embodied in QCD.

These narrow states, too low in mass to decay to $b\bar{q} + \bar{b}q$ mesons, are further expected in QCD to decay by the emission of three gluons (the smallest number allowed by a variety of selection rules). Detailed analysis of the hadronic final state of the T indicates that even this prediction is consistent with all of the observations. Thus this T system confirms dramatically the QCD model of strong interactions.

The T''' should be a prolific source of B mesons, and hence detailed studies of their properties (and those of the b quark) will be possible. A B meson has yet to be demonstrated at Cornell (an earlier possibility from a CERN experiment has since disappeared with further data). Its decay modes are many and the multiplicity of particles in each is expected to be very high, making a clean identification difficult. Nonetheless, it is possible to look for irregularities that might be associated with the T''' and hence the B particles. An excess of K mesons is observed and these are expected if the b quark (within the B meson) undergoes a weak decay transition to the charm quark, which has a well documented predilection for strange particles in its decay. The excess is, within the large errors, just what would be expected. Furthermore, the semi-leptonic weak decays of the B mesons ($B \rightarrow x\bar{\nu}$, where ν represents one or more hadrons) lead to increased numbers of electrons and muons. Such increases are observed and the inferred branching ratios for these decays are 24 ± 6 per cent, whereas the standard model, containing a top quark, would suggest a value of ~ 34 per cent, not widely inconsistent with the experimental value. Thus the data, sparse as they are at the moment, suggest that the standard model remains intact with the most likely existence of a top (charge $\frac{2}{3}$) quark and a weak decay chain $t \rightarrow b \rightarrow c \rightarrow s$. Higher energies than those achieved so far at PETRA (~ 38.6 GeV) will be needed in the search for this quark. A very recent paper (S. Glashow *Phys. Rev. Lett.* **45**, 1914; 1980) puts forward further theoretical arguments for a mass of 38 ± 2 GeV for the top quark. Experimentalists should soon be able to check on this. The present gauge model relies on its existence. The new facilities in Cornell will undoubtedly produce far more quantitative results in the future. These will allow more systematic tests of our current ideas of the quarks and their strong and weak interactions and perhaps in this quantitative exposure new possibilities will emerge. After all, we have as yet no explanation for the number of generations of quarks and leptons. Experiments will perhaps one day provide the clue. An exciting few years are in store at Cornell. □

Molecular hydrogen in T Tauri

from Stephen P. Maran

THE discovery of ultraviolet molecular hydrogen emission in T Tauri with the International Ultraviolet Explorer satellite (see Brown *et al.* this issue of *Nature* p.34) has considerable potential for three fields of research: the nature of T Tauri stars and their associated nebulae; the formation and development of the Solar System; and the study of molecular hydrogen in the Galaxy. T Tauri stars have masses about equal to that of the Sun, but are rotating much more rapidly and are thought to be stars still-in-the-making, only about a million years old and still contracting towards the Main Sequence. Some appear to be losing mass, the so-called 'T Tauri wind', while others appear to be still accreting matter from the surrounding nebulae. It is widely accepted that the Sun passed through a T Tauri phase and models of Solar System formation take into account the postulated high wind and luminosity and the concomitant decreasing gravitational attraction of the early Sun to solve a number of problems. Among them are the placing of Mercury and perhaps other planets into their present orbits, the sweeping out of excess gas and dust in the protoplanetary nebula, the dispersal (in modern versions of the Laplace nebular hypothesis) of gas rings in the planetary orbits, the formation of chondrules found in meteorites, and the reduction of the Sun's rotation to its present very low rate. T Tau itself, the prototype of the stellar class, is associated with a small luminous cloud called Burnham's nebula.

It had been thought unlikely that ultraviolet emission known to occur in nebulae would be detectable in Burnham's nebula with instruments having the sensitivities of the two IUE spectrographs. Brown *et al.* confirmed this, for they found no previously known nebular lines in the spectrum of the object, but instead discovered emission in the H_2 Lyman bands that previously were seen only in sunspots. In recent years, astronomers have concluded that the interstellar gas is not dominated simply by atomic hydrogen but that there is also a substantial molecular component. This has been largely inferred from observations of the CO molecule in the galactic plane but H_2 in the general medium has also been seen through its ultraviolet absorption lines (a technique feasible only in the directions of appropriate hot stars that serve as background continuum sources), and H_2 in several nebulae (including Burnham's nebula) has been found via the infrared

vibration bands. More recently, a bound-free absorption continuum of the H_2^+ ion has been detected in planetary nebulae (S. Heap and T.P. Stecher *Proc. Conf. on the Universe at Ultraviolet Wavelengths* 1980).

Brown *et al.* distinguish between two mechanisms for H_2 excitation in Burnham's nebula and favour the 'shock excitation' model. The nebula is much smaller than the Oort cometary cloud that surrounds the Sun, and hence phenomena on its scale may well be relevant to the early evolution of the Solar System. Since H_2 must be a principal component of the nebula, observations with more sensitive ultraviolet instruments having very fine angular resolution (notably, the High Resolution Spectrograph now under development for flight on the *Space Telescope*) will enable astronomers to investigate the dynamics of the shocked gas. In the meantime, nebular specialists will be busy calculating where else in the Galaxy this new diagnostic may be detectable. □

Planarian neoblasts

from J. Baguna

In a News and Views article on 21 August 1980 Jonathan Slack¹ reviewed briefly the sources of the cells involved in regeneration in planaria and, in doing so, made several assertions that deserve comment.

(1) 'Neoblasts' are indeed small basophilic cells present in planaria. But to think of them as cells 'reserved' for regeneration is equivocal since many planarian species, having sexual reproduction, never regenerate in nature. So what are the neoblasts for? The answer lies in considering the neoblast not just as a 'reserve' cell but as the stem cell of most differentiated cell types supplying transit cells to balance the death of cells.

(2) In planarian regeneration it is meaningless to argue whether neoblasts come from all parts of the body or from local tissues through metaplasia because the blastema can be formed (and is formed) from pre-existing local neoblasts proliferating near the wound. Moreover, Wolff's experiments², which have been cited as evidence for migratory neoblasts, have been consistently criticized³⁻⁶ because at most, they only show the 'repopulation' of the irradiated region by healthy neoblasts through cell division and do not prove a direct migration of neoblasts from unirradiated regions to the wound.

(3) Slack points out that "histological and electron microscopic studies suggest that local dedifferentiation does occur". The

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available evidence is scanty and unclear so this seems a bold assertion. Other authors^{7,8} have been unable to find any dedifferentiating features in any of the blastema constituting cells.

(4) The results reported by Gremigni and Miceli⁹ supporting cell dedifferentiation and transdifferentiation in planarians, though offering new ways to re-evaluate the problem of blastema formation, are not as conclusive as has been suggested. First of all, the sequence proposed: germ cell → blastema cell → muscle cell, and its corollary of dedifferentiation and metaplasia are not necessarily related as the loss of an haploid complement during spermiogenesis, though one of the first steps from neoblasts to male germ cells, is only a trivial phenomenon regarding cell determination. Unless spermatids or primary spermatocytes are shown to give rise to blastema cells, and then to differentiated cells, we cannot talk properly of dedifferentiation and metaplasia. At the most, this would qualify for dedetermination (or trans-determination) and the sequence proposed should be read instead as predetermined or determined: germ cell → blastema cell → muscle cell. Second, and more important, the sequence suggested by Gremigni and Miceli, if proved correct, has to be demonstrated for other differentiated cell types since several planarian species, being asexual, lack germ cells. Besides, it is possible, as suggested by Betchaku⁴ and Pederson⁸, that both processes (neoblasts and metaplasia) occur during planarian regeneration. If this were the case, the important issue would be to know the partitioning between both phenomena during planarian regeneration.

(5) Though most (but not all, witness *Hydra*) regenerative phenomena in the Animal Kingdom have been proved to occur through cell dedifferentiation and re-differentiation, the present trend to extrapolate this mechanism to all animal groups (including planarians) overlooks the possible relationship between the actual mechanism of regeneration and the complexity of the species. Planarians are one of the most primitive acoelomates, and their tissular complexity is rather low if compared with other epimorphic-regenerating organisms like Amphibia or Insecta. No wonder that dissimilar mechanisms may operate during regeneration in these groups. The planarian neoblast system, based on a unique and multipotential self-renewing stem cell, though appropriate to the low-level complexity of planarians, is clearly

inadequate for organisms with static (decaying) cells and transit cells that require different determined stem cells placed in different body regions. Consequently, the dedifferentiation process, necessary for Amphibia regeneration, is not necessary for organisms like planarians which are in a continuous state of rapid cell turnover¹⁰. Unless new data on planarian histology and cytology, cell dynamics, and life cycle are obtained, the extrapolation of other mechanisms of regeneration to planarians^{1,9} and the construction of theoretical models of planarian regeneration¹¹ will be of little value.

J.M.W. Slack comments: In my article I attempted to show that several *independent* issues are involved in establishing the source of cells for regeneration, each of which must be approached through a different experiment. As Baguña implies, the simple dichotomy — 'neoblasts versus metaplasia' — is often not an adequate statement of the problem. I believe that the controversy which has arisen in this field in the past has largely been caused by an imprecision in the question asked. □

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A carbohydrate signal for intracellular transit

from Mike Geisow

JUST over a year ago, the function of the oligosaccharide chains added to many newly synthesized proteins was discussed in these columns (*Sugars and intracellular recognition*, *Nature* **281**, 15; 1979). It was noted that an early hypothesis — that carbohydrate acted as a marker for secretion — had been superseded by the discovery of the signal peptide. However, it did seem as if carbohydrate on non-secreted proteins might still act as a signal for intracellular transit from the endoplasmic reticulum to subcellular, membrane-enclosed destinations. Recent work has shown that this is indeed the case for lysosomal enzymes.

Characteristically, lysosomal enzymes are glycoproteins and many have been shown to contain mannose residues phosphorylated at carbon-6 in the hexose rings. Both glycosylation and phosphorylation occur in the endoplasmic reticulum and appear to be directed by the sequence and conformation of the polypeptide substrate; not all proteins receive both modifications. The importance of both the

mannose and the phosphate groups in receptor-mediated processes first became apparent when glycoproteins expressing both determinants were shown to be rapidly and specifically endocytosed by fibroblasts. Next, the uptake of a variety of lysosomal hydrolases was found to be subject to competitive inhibition by mannose phosphate. These observations suggested three rival routes for lysosomal enzymes from their sites of synthesis to primary lysosomes. Two of these, involving secretion followed by re-uptake or cycling as receptor complexes via the surface membrane, appear to be of minor importance: receptor-saturating levels of extracellular mannose-6-phosphate do not deplete lysosomal enzyme levels. Fischer and his colleagues (*J. biol. Chem.* **255**, 9608; 1980) now present firm evidence for a major intracellular transit system using β -hexosaminidase B in fibroblasts.

It was found that 80 per cent of the mannose-6-phosphate receptors were intracellular and faced the interior of endoplasmic reticulum or lysosomes. The highest specific activity of enzyme-binding receptors was present in these cell fractions. Moreover, the intracellular receptors were combined with endogenous enzymes and presented an occupancy gradient which ran steeply downhill from endoplasmic reticulum to lysosomes. The inference is that proteins which receive phosphorylmannose in the endoplasmic reticulum as a result of as yet unknown conformational signals in the polypeptide, are recognized and transferred to primary lysosomes by an essentially intracellular route.

In the report referred to earlier, attention was drawn to the frequent observation of secretory protein proteolysis in endocrine cells treated with inhibitors of protein glycosylation. In such cells, failure of the glycosylation system presumably produces not only non-glycosylated hormones, but also carbohydrate-free acid hydrolases co-synthesized in the endoplasmic reticulum. What happens to the non-glycosylated lysosomal enzymes? In I-cell disease, defective addition of phosphorylated carbohydrate to the enzymes results in their secretion. Co-packaging of acid hydrolases and normal secretory products in the acid environment of a secretion granule may well explain the observed aberrant proteolysis. This argument leads to an interesting thought — is such proteolytic attack on hormones always abnormal? Some hydrolases appear to be naturally present in secretion granules as opposed to lysosomes. These are the conversion enzymes which give rise to products as diverse as insulin, oxytocin and enkephalins from the original precursors. Do such proteases arrive at their destination in the secretory granule because they normally lack a mannose-6-phosphate-containing oligosaccharide chain? □

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Beyond the giga-seal

from Robert McBurney

Two recent technical developments promise a new era in the examination of the movements of ions across the surface membranes of excitable cells. As well as the achievement of much higher current resolutions it is expected that one technique, using an 'excised patch', will make it possible to monitor changes in membrane properties while being able to directly influence the environment on the inside surface of the cell membrane.

The new techniques stem from the development by Neher and Sakmann (*Nature* **286**, 71; 1980) of a method for the direct measurement of the ionic current flowing through single ion channels in biological membranes. These recordings of picoampere (10^{-12} A) currents are made with a specially constructed glass pipette which seals off a small patch of membrane (about $1 \mu\text{m}$ in diameter) on the surface of a cell from the rest of the surface membrane. The successful interaction of agonist molecules in the solution inside the pipette with receptor-ion channel complexes on the patch of membrane generates discrete pulses of ionic current which are recorded by a low-noise current-to-voltage converter. The current loop for measurements is from the pipette internal solution through open ion channels in the patch to the cytoplasm, then through 'leakage' channels in the membrane outside the patch to the bulk bathing solution and through the recording device to the pipette solution.

If some of the current flowing through the ion channels fails to complete this recording circuit, and a proportion returns via the seal between the pipette wall and membrane or through leakage channels in the patch itself, the true single-channel current amplitude is not measured. While the latter return pathway is not significant, except for larger patches on very small cells, the achievement of adequate seals has been a key factor in the application of this technique to different preparations. Not only does the relationship between seal resistance (R_{seal}) and recording system resistance determine the proportion of the actual current recorded but the thermal current noise in the seal resistance (inversely proportional to $\sqrt{R_{\text{seal}}}$) determines the lower limit of current resolution of the recording system.

Despite problems in creating adequate seals, the patch recording technique has been successfully applied over the past five years to a variety of preparations, including denervated frog muscle, cultured rat muscle (myoballs), locust muscle and

squid giant axons. The channels investigated have mostly been those activated by receptor agonists (cholinergic in frog and rat muscle; glutaminergic in locust muscle), although in one case (squid giant axon) the properties of voltage-dependent K^+ channels have been studied. The technique has been used to confirm and extend data obtained by the analysis of membrane current fluctuations (see Stevens *Nature* **270**, 391; 1977). In addition, it has yielded new information concerning the detailed kinetics of action of some drugs, for example local anaesthetics on acetylcholine-activated ion channels (Neher and Steinbach *J. Physiol., Lond.* **277**, 153; 1978), and has revealed the behaviour of ion channels during desensitization (Sakmann *et al.* *Nature* **286**, 71; 1980).

The first exciting new development to come from Neher and Sakmann's technique arose from efforts to increase the sealing resistance. By applying suction to the recording pipette as it was placed on the surface membrane of a cultured muscle cell, F. Sigworth and E. Neher (Göttingen) found that a very high-resistance seal ($>10^9 \Omega$) was, in some cases, suddenly created. This seal and its associated low current-noise even at a bandwidth from DC-7 kHz allowed Sigworth and Neher to resolve single voltage-dependent Na^+ channels: those normally associated with the generation of muscle action potentials. In their preliminary report (*Nature* **187**, 447; 1980), as well as describing the 'giga-seal' and presenting clear records of single Na^+ channels, they have shown that these channels have conductance and average lifetime values which agree with those obtained by more indirect means. They also demonstrate the independence of channel behaviour by showing that the sum of many records, usually containing just one channel opening in response to a fixed membrane depolarization, mimics the behaviour of many channels operating simultaneously.

The future application of the 'giga-seal' technique to excitable tissues will permit the investigation of 'gated' ion channels in cells or regions of cells not amenable to voltage-clamp analysis with intracellular microelectrodes, such as smooth cells, gland cells, unmyelinated axons and possibly even nerve terminals. In addition, the technique provides improved current resolution and, for single-channel currents already observed by the original method, a much wider bandwidth for recording which will undoubtedly reveal finer details in the kinetics of channel activation and inactivation.

Perhaps more exciting than the achievement of the 'giga-seal' itself has

been the recent observation made by two groups of workers, R. Horn and J. Patlak (Yale) and O. Hamill and B. Sakmann (Göttingen). They found that, once established, this seal was almost impossible to shake off. When the electrode was pulled away from the cell it seemed that the patch of membrane was excised on, or within, the tip of the electrode and single-channel currents could still be observed if the transmembrane voltage was appropriately adjusted. After a short time the recording ceased, possibly due to a membrane-bound vesicle being formed within the electrode tip (because the high-resistance seal remained). The two groups solved the problem of vesicle formation in different ways: Horn and Patlak (*Proc. natn. Acad. Sci. U.S.A.* **77**, 6930; 1980) found that fluoride ions delayed or prevented the process; while Hamill and Sakmann (*J. Physiol., Lond.* in the press) found that by lifting the electrode through an air-water interface they were able to re-expose the patch.

In their preliminary report Horn and Patlak illustrate one of the special features of this preparation: they examine the dependence of the conductance of acetylcholine-activated channels on sodium ion concentration in a situation where the latter could be defined on both sides of the membrane. Such access permits a vast array of experiments to be undertaken with controlled environments on either side of the membrane, similar in principle to the study of ion transport in 'doped' artificial lipid membranes, and these will certainly result in a clearer idea of the mechanism of ion transport and selectivity of natural ionophores.

However, the most important aspect of the preparation may simply prove to be access to the inside surface of cell membranes. It will now be possible to investigate directly the action of intracellular messengers (for example, cyclic nucleotides, Ca^{2+}) and cellular metabolic products on membrane excitability and transport processes. It is hoped that this 'excised patch' technique will be generally applicable to cells derived from a wide range of tissues. For example, its application to heart cells will certainly provide an excellent preparation with which to study the relationships between 'second messengers', and protein phosphorylation and ion channels in the hormonal modulation of membrane conductance changes underlying cardiac pacemaker activity. □

Erratum: In *Antimatter back to front* by C. H. Llewellyn Smith (*Nature* **289**, 534 February 12, 1981) the last sentence in the first paragraph should have read "The discovery of CP violation by Christenson, Cronin, Fitch and Turlay in 1964, for which Cronin and Fitch were awarded the 1980 Nobel prize, was therefore almost as great a shock as the discovery of parity violation".

Corrigendum: In *Cement — a respectable material?* by D. D. Double (**289**, 348, January 29, 1891) the figure of "100 MN m⁻²" quoted as the modulus of mother-of-pearl shell should have been "about 60 GN m⁻²".

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ARTICLES

Mesoscale convection in the clouds of Venus

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The cellular structures seen in UV images of the atmosphere of Venus probably represent convection cells. If so, this shape (horizontal dimensions 10–100 times greater than vertical dimensions) cannot be accounted for by radiation transfer alone but requires anisotropic eddy diffusivity.

UV PICTURES of Venus taken by the Mariner 10 and Pioneer Venus spacecraft show cellular structures near and downwind of the subsolar point at cloud top heights of 60–70 km (Fig. 1)^{1–3}. These structures may result from various eddy phenomena, for example, wave trains intersecting to form a criss-cross pattern. However, the most natural interpretation, given their proximity to the subsolar point, is that they are atmospheric convection cells visible at the cloud tops.

Cloud level convection

The presence or absence of an adiabatic layer at the cloud tops would be strong evidence either for or against convection in this region of the atmosphere. Unfortunately, the four Pioneer Venus probes entered the atmosphere away from the subsolar point, at night or in the early morning. They, nevertheless, all measured an adiabatic temperature gradient between ~50 and 55 km altitude with stable layers above and below⁴. We therefore regard the UV cellular features as evidence for convection in the cloud-top subsolar region and suggest that the cloud level adiabatic layer in this region is either higher or thicker than the one measured by the probes, so as to encompass the cloud tops⁵. For example, in the 55–60 km altitude range the static stability measured by the probes has a mean value of $2\text{--}3\text{ K km}^{-1}$. This 5-km thick layer of the atmosphere could become adiabatic if the temperature were increased by 10–15 K between morning and afternoon. Then the adiabatic layer would extend to 60 km, the lower limit for the altitude of the UV features. The required heating is compatible with a diurnal temperature cycle involving an atmospheric superrotation rate of 5 days⁶ and a radiative cooling time of ~100 days⁷; which represents a temperature variation of ~5%. Alternatively the cellular features may be located in an adiabatic layer above 65 km altitude (where static stability data do not exist)⁵. We might also be seeing penetration of lower lying convection into an overlying stable cloud top region².

An adiabatic cloud top layer is likely to be bounded above and below by regions of high static stability caused by radiative cooling of the cloud tops and radiative heating of the cloud base⁸. The depth of the convecting layer is then of the order of 10 km. Given the horizontal scale of the cellular UV features, $10^2\text{--}10^3\text{ km}$, this implies aspect ratios (diameter/depth) of the order of $10\text{--}10^2$. This is several times the aspect ratio of mesoscale cellular atmospheric convection on Earth⁹, which in turn is several times the aspect ratio obtained in laboratory and theoretical studies of cellular convection at moderately supercritical Rayleigh numbers¹⁰.

We now present a theory to explain these high aspect ratios for cloud level convection in the Venus atmosphere. Mesoscale atmospheric convection on Earth takes place immediately above the planetary surface and it is, therefore, affected by the

very steep temperature gradient in the thermal boundary layer¹¹. The properties of the cloud level Venus atmosphere together with the large aspect ratio of the cells require that the thermal diffusivity due to small-scale eddies be large and anisotropic.

Radiative and eddy processes

The major difference between laboratory and atmospheric convection is that in the latter radiative and eddy processes play a major part in the transport of heat and momentum. To represent the role of eddies an eddy viscosity ν and an eddy thermal diffusivity κ are used. These eddy diffusivities may be anisotropic. To account for this, we let ν and κ represent the diffusivities in the vertical direction and $m\nu$ and $m\kappa$ the horizontal diffusivities. Thus we assume that m , the ratio of horizontal to vertical diffusivity, is the same for momentum and heat transport. To account for radiative heat transfer within the convecting fluid, we linearize the Boussinesq equations for a compressible atmosphere with an internal heat source¹² Q about a basic state in radiative equilibrium. The incremental

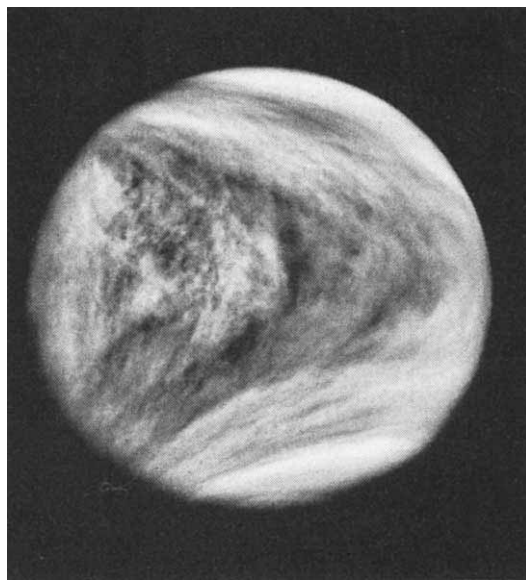


Fig. 1 UV image of Venus from the Pioneer Venus Orbiter Cloud Photopolarimeter². The rotation of the atmosphere is from right to left. Cellular structures may be seen in the equatorial region, near and downwind of the subsolar point.

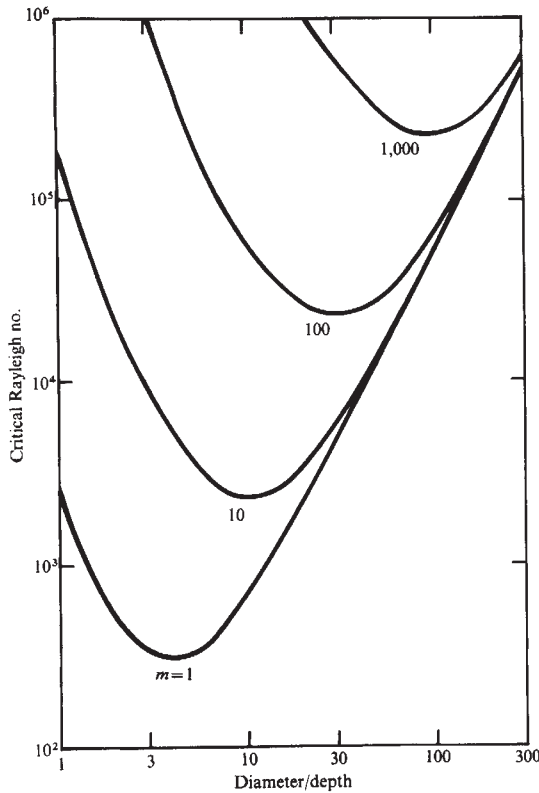


Fig. 2 Critical values of the Rayleigh number for the onset of convection as a function of aspect ratio, for different values of eddy anisotropy ratio m . For these curves $\tau = 10$ and $\gamma = 1$, as implied by $\kappa = 10^6 \text{ cm}^2 \text{ s}^{-1}$.

heating in the perturbed state Q' is approximated by the newtonian cooling law

$$Q'/\rho c \approx -\theta^*/\tau^* \quad (1)$$

where ρ is the density, c is the specific heat at constant pressure, θ^* is the perturbation temperature, and τ^* is the radiative cooling time. The newtonian cooling approximation is valid at cloud levels in the Venus atmosphere, and values of τ^* have been obtained⁷ at different altitudes from detailed radiative transfer calculations.

For simplicity we assume that the thermal expansion coefficient α , the basic state temperature gradient dT_0/dz^* (z^* is altitude), and the adiabatic temperature gradient Γ_{ad} are all constant. Then the governing equations for θ^* , perturbation velocity $\mathbf{u}^*$, and perturbation pressure π^* are

$$\left(\frac{\partial}{\partial t^*} - m\nu\Delta_2^* - \nu\frac{\partial^2}{\partial z^{*2}}\right)\mathbf{u}^* = -\nabla^*\pi^* + g\alpha\theta^*\mathbf{k} \quad (2)$$

$$\nabla^* \cdot \mathbf{u}^* = 0 \quad (2)$$

$$\left(\frac{\partial}{\partial t^*} + \frac{1}{\tau^*} - m\kappa\Delta_2^* - \kappa\frac{\partial^2}{\partial z^{*2}}\right)\theta^* = \left(\left|\frac{dT_0}{dz^*}\right| - \Gamma_{ad}\right)(\mathbf{k} \cdot \mathbf{u}^*) \quad (4)$$

In the above, $-g\mathbf{k}$ is the acceleration of gravity and Δ_2^* is the laplacian with respect to horizontal coordinates. We have assumed a motionless basic state and have thus neglected any wind velocity gradients. The zonal wind at the cloud tops is large but its vertical shear is unknown⁵. The fact that the putative convective features are cells instead of rolls argues that the wind shear is small enough to be neglected³.

We remove dimensions from equations (2)–(4) using the depth of the fluid d as the length scale, d^2/κ as the time scale, and $(|dT_0/dz^*| - \Gamma_{ad})d$ as the temperature scale. This gives

$$\left(\frac{1}{P}\frac{\partial}{\partial t} - m\Delta_2 - \frac{\partial^2}{\partial z^2}\right)\mathbf{u} = -\nabla\pi + R\theta\mathbf{k} \quad (5)$$

$$\nabla \cdot \mathbf{u} = 0 \quad (6)$$

$$\left(\frac{\partial}{\partial t} + \frac{1}{\tau} - m\Delta_2 - \frac{\partial^2}{\partial z^2}\right)\theta = \mathbf{k} \cdot \mathbf{u} \quad (7)$$

where $\mathbf{u}$, π , θ , t , z , ∇ , and Δ_2 are dimensionless quantities and operators. Physical processes within the fluid are then determined by four nondimensional parameters: m , the Prandtl number

$$P \equiv \nu/\kappa \quad (8)$$

the Rayleigh number

$$R \equiv \frac{\alpha g(|dT_0/dz^*| - |\Gamma_{ad}|)d^4}{\nu\kappa} \quad (9)$$

and the dimensionless radiative cooling time

$$\tau = \tau^*\kappa/d^2 \quad (10)$$

We must also account for the effect of radiative transfer at the boundaries of the convecting fluid. At the upper boundary, the additional heat conducted upwards in the fluid $-\rho c \kappa d\theta^*/dz$ must equal the extra heat radiated to space $4\sigma T_0^3\theta^*$. A similar condition applies at the lower boundary. Thus, in nondimensional form,

$$\frac{d\theta}{dz} \pm \gamma\theta = 0 \quad \text{at } z = \pm \frac{1}{2} \quad (11)$$

where

$$\gamma = \frac{4\sigma T_0^3 d}{\rho c \kappa} \quad (12)$$

We assume free boundaries as appropriate for a convecting layer within an atmosphere. The velocity boundary conditions are¹⁰

$$\mathbf{u} \cdot \mathbf{k} = \frac{d^2}{dz^2}(\mathbf{u} \cdot \mathbf{k}) = 0 \quad \text{at } z = \pm \frac{1}{2} \quad (13)$$

Two special cases illustrate the different roles of radiative transfer within the fluid and at the boundaries. In the limit $\gamma \rightarrow \infty$, equation (11) becomes the isothermal condition $\theta = 0$ commonly used in convection theory. This case of perfectly conducting boundaries neglects the role of radiation in exchanging heat at the boundaries. The effect of the τ^{-1} term, which represents radiative transfer within the fluid, is then to increase the stability of the fluid and to decrease the aspect ratio of convection cells. This result was also obtained by Goody¹³, who incorporated the equations of radiative transfer into the formulation of the convection problem but used the boundary condition $\theta = 0$. The stabilizing effect occurs because radiative

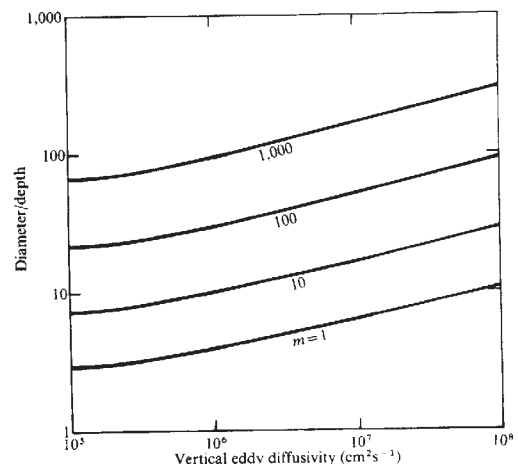


Fig. 3 Aspect ratio for the minimum Rayleigh number, as a function of κ , for various values of m .

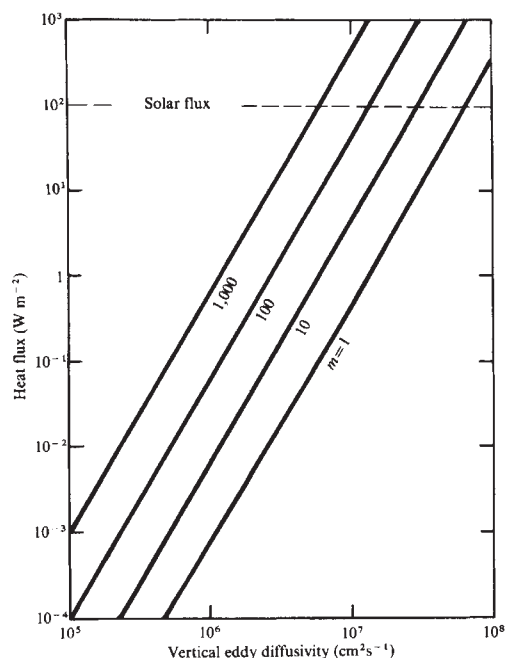


Fig. 4 Minimum convective heat flux required to sustain convection for the indicated values of κ and m .

transfer adds to the effect of conduction in equalizing temperature differences between different parts of the fluid. The decrease in aspect ratio occurs because radiative transfer, unlike conduction, does not depend on the proximity of hot and cold fluid elements. In ordinary convection problems, cells with low aspect ratios are not seen because the proximity of hot upwelling regions and cold downwelling regions leads to large horizontal conductive heat transfer. When radiative transfer carries a significant amount of heat, this effect becomes less important and the cells tend to have lower aspect ratios.

The limit $\tau \rightarrow \infty$ accounts for radiative transfer at the boundaries but not within the bulk of the convecting fluid. As γ decreases the fluid becomes less stable and the aspect ratio of convection cells increases^{11,14,15}. The smaller γ is, the less efficient is radiation in exchanging heat at the boundaries. This explains the decreased stability of the radiative-convective static state. Fluid parcels must also spend more time at the boundaries exchanging heat, and this results in larger aspect ratios.

Thus, radiative transfer at the boundaries and within the fluid have opposite effects on the aspect ratio. In addition, anisotropic diffusivities with $m > 1$ tend to increase the aspect ratio^{16,17}. To see which effect 'wins' we solve the boundary value problem for realistic parameter values. Except for the eddy diffusivities, these are known at least in order of magnitude: $d \sim 10$ km; at 60 km altitude $c = 8.6 \times 10^6$ erg g⁻¹ K⁻¹, $\rho = 0.5 \times 10^{-3}$ g cm⁻³, and $T_0 = 270$ K from *in situ* probe measurements⁴; at cloud levels $\tau^* \sim 10^7$ s according to radiative transfer calculations⁷. Using these values, equations (10) and (12) give

$$\tau = \kappa / (10^5 \text{ cm}^2 \text{ s}^{-1}) \quad (14)$$

$$\gamma = (10^6 \text{ cm}^2 \text{ s}^{-1}) / \kappa \quad (15)$$

Thus, choosing the vertical thermal diffusivity κ determines τ and γ . The boundary value problem can then be solved by standard methods¹⁰. The onset of convection is steady for finite γ (ref. 11) and the additional effects of finite τ and $m \neq 1$ should not change this result. Thus we set $\partial/\partial t = 0$ in equations (5)–(7). We assume a horizontal wavenumber a and obtain an eigenvalue problem that determines R , the Rayleigh number for the onset of convection, as a function of a .

Figure 2 shows R plotted against the aspect ratio ($2\pi/a$) for different values of m , obtained by numerical solution of the eigenvalue problem with $\kappa = 10^6 \text{ cm}^2 \text{ s}^{-1}$. To match the cell dimensions observed in the Venus atmosphere, the minimum R must occur for aspect ratios of 10–100. Figure 2 shows that this occurs for $\kappa \sim 10^6 \text{ cm}^2 \text{ s}^{-1}$ and $m \sim 100$. These are magnitudes commonly used for the terrestrial atmosphere^{16–18}. Thus our theory can explain the high aspect ratios using reasonable parameter values.

There are, of course, many combinations of κ and m which give the desired aspect ratios. Figure 3 plots the aspect ratio at minimum R as a function of κ . There is a trade-off between m and κ : a lower eddy anisotropy is allowed if the magnitude of the eddy diffusivity is larger. For example, $m \sim 10$ and $\kappa \sim 10^7 \text{ cm}^2 \text{ s}^{-1}$ will also give aspect ratios in the range 10–100. Figure 3 also shows the influence of radiative transfer on the aspect ratio. For $\kappa \leq 10^5 \text{ cm}^2 \text{ s}^{-1}$ the aspect ratios approach the values one would obtain by neglecting radiative transfer ($\gamma = \tau = \infty$). For larger values of κ there is a noticeable effect: the boundary radiation effect (γ) dominates the internal radiation effect (τ), so that the aspect ratio is increased. Larger values of κ imply that eddies transfer heat very efficiently within the fluid. At the boundaries, heat is transferred by radiation because eddies are suppressed in the stable layers which bound the convecting region. This radiative transport is comparatively inefficient, causing larger aspect ratios as explained above.

The heat transport due to convection provides an important constraint on the size of κ . For instance, using $\kappa = 10^6 \text{ cm}^2 \text{ s}^{-1}$ and $m = 100$ we find a minimum Rayleigh number of 2.27×10^4 (Fig. 2). Using $\nu = \kappa$ we find

$$|dT_0/dz^*| - \Gamma_{ad} = \nu \kappa R / \alpha g d^4 = 7 \times 10^{-4} \text{ K km}^{-1} \quad (16)$$

This is the minimum superadiabaticity required to sustain convection in this case. It implies a minimum convective heat flux

$$\rho c \kappa (|dT_0/dz^*| - \Gamma_{ad}) = 6 \times 10^{-2} \text{ W m}^{-2} \quad (17)$$

Increasing κ will rapidly increase the heat flux to values greater than the downward solar radiative flux at cloud levels (estimated at 10^2 W m^{-2} from Pioneer Venus probe data¹⁹), a non-physical result. Figure 4, displaying the results of calculations analogous to equations (16) and (17) for different m and κ , shows that this limit is reached for $\kappa \geq 10^8$ when $m = 1$. Thus the combination $m = 1$, $\kappa \geq 10^8$ is ruled out even though it provides the correct aspect ratios (Fig. 3).

Conclusions

Our results show that the apparent flatness of large-scale convection cells in the Venus clouds can be explained by anisotropic eddy diffusion and radiative transfer effects. Horizontal eddy diffusivities must be at least 10-fold greater than vertical diffusivities. Anisotropy ratios $\geq 1,000$ are sufficient to account for the flattening in cases where the vertical eddy diffusivity is $\geq 10^5 \text{ cm}^2 \text{ s}^{-1}$ and the effects of radiative transfer are negligible. For vertical diffusivity $\geq 10^5 \text{ cm}^2 \text{ s}^{-1}$ radiative transfer contributes to the flattening. But radiative transfer alone cannot account for the apparent aspect ratios; this would require vertical heat diffusivities large enough to give a non-physically high value of convective heat transport.

The present theory is greatly simplified by the linearization of the equations about a static basic state. We have not only assumed small velocity perturbations but we have also made the Boussinesq approximation, which assumes small density variations. The latter is strictly valid only when the depth of the convecting layer is smaller than a scale height¹² (6 km in the region of interest). Finally, we have accounted for radiative heating within the convecting layer and have found that this effect tends to reduce the aspect ratio of the cells. Yet we cannot rule out the possibility that in fully developed convection the opposite effect occurs. In fact, Tritton has proposed on the basis of laboratory studies that internal heating contributes to the flattening of the convection cells on Venus²⁰. Considerations

such as this underscore the importance of studying nonlinear effects.

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The structure of eight distinct cloned human leukocyte interferon cDNAs

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Eight classes of human leukocyte interferon (LeIFN) cDNA clones have been identified in a cDNA library prepared from a myeloblastoid cell line. The nucleotide sequences demonstrate that the multiple human LeIFN genes code for a family of homologous, yet distinct proteins. One of the cDNA clones may have been derived from the transcription of a LeIFN pseudogene.

INTERFERONS are a family of proteins which confer viral resistance in target cells^{1,2} as well as inhibiting cell proliferation and modulating immune response (see refs 2, 3 for reviews). Human interferons were originally classified by their respective cells of origin. The major interferons produced in leukocytes (LeIFN or IFN- α) and in fibroblasts (FIFN or IFN- β) have been shown to be antigenically distinct, acid-stable molecules collectively designated 'classical' or type I interferons. Intense clinical interest in IFN coupled with the inability to obtain large amounts of the purified protein from human cells has spurred efforts to produce high yields of human interferon in the bacterium *Escherichia coli* by recombinant DNA procedures. Several groups have recently reported the bacterial synthesis of various interferons^{4–9}, and the efficacy of such material in limited animal testing has been demonstrated⁵.

We recently described the construction of a collection of cDNA clones prepared using 12S RNA isolated from a human myeloblastoid cell line⁵. Several cDNA clones were identified by hybridization with synthetic DNA probes designed from partial amino acid sequence information of human leukocyte interferon. The cDNA insert of one of the hybrid plasmids (pL31) was sequenced and used to construct a plasmid which directed the synthesis of large amounts of a biologically active human LeIFN, termed LeIFN A (ref. 5). Restriction endonuclease analysis showed extensive differences between the additional hybridizing clones, suggesting that several different human LeIFNs might be expressed by the myeloblastoid cell line KG-1 (ref. 10). Furthermore, pL31 differs considerably from the human LeIFN cDNA clone described by Weissmann and colleagues⁴ in both nucleotide sequence and deduced amino acid sequence; yet both of the encoded LeIFNs show antiviral activity^{4,5}.

Additional evidence for the existence of a multigene family of human LeIFNs is found in the heterogeneity of the IFN proteins prepared from human leukocytes¹¹ and lymphoblasts^{12,13}. NH₂-terminal amino acid sequences^{12–14} and amino acid sequences of tryptic and chymotryptic peptides¹³ have revealed at least five

distinct, but homologous, LeIFNs. At least eight distinct chromosomal LeIFN genes have recently been isolated from a human gene library (ref. 15, and R.L. and A.U., unpublished results). In contrast, there is no evidence for multiple human FIFN genes. NH₂-terminal amino acid sequences of FIFN determined by two different groups^{16,17} agree with results obtained from nucleotide sequencing of cloned FIFN cDNAs^{7,18,19} and FIFN mRNA^{20,21} in suggesting that there may be only one gene for FIFN. We describe here genomic hybridization data consistent with the existence of about ten human LeIFN genes and a single FIFN gene, and present the DNA sequences of eight distinct LeIFN cDNA clones identified in a cDNA library prepared from the myeloblastoid cell line KG-1.

Evidence for multiple LeIFN genes

To estimate the number of LeIFN genes in the human genome, human spleen DNA was digested with various restriction endonucleases, electrophoresed through agarose gels and transferred to nitrocellulose paper²². Hybridizations were done using radiolabelled LeIFN and FIFN⁷ cloned cDNAs (Fig. 1). The number of LeIFN genes can be estimated from the number of hybridizing bands in the lanes containing *Hind*III and *Taq*I digests. As none of the LeIFN cloned cDNAs contains either of these restriction sites (see below), and because the human LeIFN genes do not seem to have any intervening sequences (ref. 15, and R.L. and A.U., unpublished results), it is likely that each band corresponds to at least one gene. Therefore, the hybridization patterns suggest the presence of at least ten LeIFN genes and only one FIFN gene. Identical patterns were obtained for the LeIFN genes regardless of whether only LeIFN A cDNA was used as the radiolabelled probe or several different types of LeIFN cDNAs were used as a mixed probe. The fibroblast interferon gene contains an internal *Bgl*II site^{7,18,19}. Only one hybridizing band was obtained for FIFN in the *Bgl*II digest because the hybridization probe was an isolated *Bgl*II-*Pvu*II fragment from cloned FIFN cDNA (ref. 7).

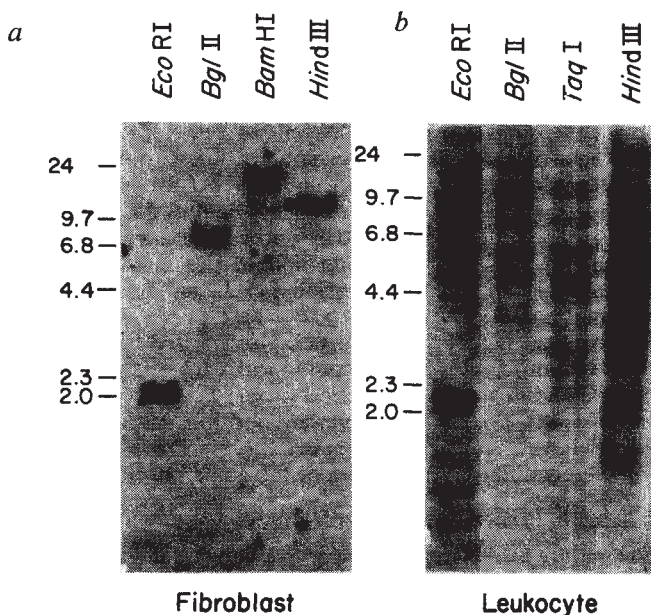


Fig. 1 Hybridization of human DNA with interferon cDNA probes. Human spleen DNA was isolated by the procedure of Blin and Stafford⁴³ and digested with the indicated restriction endonucleases. Samples (5 μ g) were electrophoresed in a horizontal 0.8% agarose slab gel and transferred to nitrocellulose filter paper²². Isolated DNA fragments consisting of *a*, a *Bgl*II-*Pvu*II fragment from cloned human FIFN cDNA (ref. 7) or *b*, LeIFN A, B, C and E interferon cDNA sequences were ³²P-labelled to a specific activity of $\sim 10^8$ c.p.m. μ g⁻¹ by random calf thymus DNA fragment primed replication⁴⁴. Hybridizations were done using 5 $\times 10^6$ c.p.m. of probe at 42 $^{\circ}$ C in 50% formamide, 5 $\times$ SSC, 5 $\times$ Denhardt's reagent⁴⁵, 50 mM sodium phosphate buffer, pH 6.5, 200 μ g ml⁻¹ denatured salmon sperm DNA and 10% dextran sulphate. After 24 h, the filters were washed at 60 $^{\circ}$ C in 0.1 $\times$ SSC, 0.1% SDS as described elsewhere²⁷. Sizes in kilobase pairs (kb) of bacteriophage λ DNA digested with *Hind*III and included in the gels as size standards are indicated. All the LeIFN cDNAs described in this paper readily cross-hybridize so that hybridization patterns similar to those in *b* have been obtained with single LeIFN cDNA probes. Two additional *Bgl*III fragments of ~ 2.0 and 1.5 kilobase pairs hybridize to LeIFN cDNA probes but are not discernible in this figure.

Identification and classification of multiple LeIFN cDNA clones

To investigate the multiplicity of cloned human LeIFN cDNAs, an *in situ* colony screening procedure²³ was carried out on 4,000 individual colonies of our cDNA library. About 50 clones hybridized to a 260-base pair *Bgl*II fragment from pL31 encoding amino acids 61–150 of LeIFN A. Of these, 33/50 contained cDNA inserts of 700 base pairs or longer and were selected for further analysis. The cDNA inserts were mapped using the restriction enzymes *Pvu*II, *Bgl*II and *Eco*RI, because *Pvu*II and *Bgl*II had been found to cleave LeIFN A cDNA⁵ and

all three cut the LeIFN cDNA cloned by Nagata *et al.*⁴. These restriction endonuclease maps allowed the division of the LeIFN cDNAs into eight groups (A to H, see Fig. 2). The largest cDNA inserts in each group were sequenced by a combination of the Maxam-Gilbert chemical procedure²⁴ and the dideoxy-chain termination procedure using the bacteriophage M13 cloning vector mp7²⁵. It was possible that different LeIFN cDNAs would display the same restriction enzyme map. Because the 3'-noncoding regions of the eight sequenced cDNA clones showed much less homology than their respective coding portions, partial sequencing was done on the 3' regions of several additional cDNAs (five cDNAs each of LeIFN A and LeIFN D, and all the cDNAs in the remaining six groups). This analysis revealed that one of the cDNA clones originally classified as type C was actually a LeIFN H-type cDNA clone which did not extend to the single 5' *Pvu*II site, giving a total of two LeIFN H cDNA clones. However, as discussed in more detail below, the nucleotide sequences of these two LeIFN H cDNA clones differ at three positions.

The restriction enzyme analysis had shown that 14 of the 33 LeIFN cDNA clones were of type LeIFN A and 9 were LeIFN D. No variations in sequence were found among the five LeIFN A cDNAs or the five LeIFN D cDNAs which were partially sequenced—this suggests that the restriction maps are indicative of distinct classes of LeIFNs. LeIFNs A and D were the only groups represented more than twice in the collection of LeIFN cDNA clones. This result was not an artefact due to use of LeIFN A cDNA as the original hybridization probe; no additional LeIFN cDNA clones were found when the 4,000 colonies of the cDNA library were tested again using mixed LeIFN A, B, C, E and F cDNA probes.

The complete sequences of the longest cDNA inserts for each of the eight classes of LeIFN are shown in Fig. 3. Pairwise comparisons of these sequences show that homologies in the coding regions range from 84 (LeIFN B versus LeIFN E) to 94% (LeIFN C versus LeIFN F). The DNA sequences were used to predict the primary protein structure of the different human LeIFNs (Fig. 4). The cDNA clones for LeIFN types A, B, C, D, F and H contain complete coding sequences for the 23-amino acid precursor signal peptide^{4,5} and the 166 amino acids of the mature LeIFNs (165 amino acids for LeIFN A, which has a deletion at position 44). The 5' end of the LeIFN E cDNA begins in the signal peptide region, and also contains an extra base pair at position 187 (Fig. 3). LeIFN G is not a full-length cDNA copy of LeIFN mRNA and contains an incomplete coding sequence.

LeIFN D has the same restriction pattern as the LeIFN cDNA clone described by Nagata *et al.*⁴. The only difference between the two clones in predicted amino acid sequence is at position 114 where valine in LeIFN D replaces alanine²⁶. Weissmann and co-workers⁶ have also recently sequenced a partial-length LeIFN cDNA clone of a second type (which they named α_2) which differs from LeIFN A only at the codon for amino acid 23 (arginine in α_2 ; lysine in LeIFN A).

Table 1 Pairwise comparisons of differences in coding sequences of human LeIFNs

	A	B	C	D	E	F	G	H	
A		31 (19)	31 (19)	29 (17)	41 (25)	30 (18)	18* (14)	28 (17)	Mature LeIFNs
B	7 (30)		32 (19)	38 (23)	45 (27)	31 (19)	23* (17)	29 (17)	
C	7 (30)	8 (35)		32 (19)	41 (25)	18 (11)	21* (16)	23 (14)	
D	6 (26)	9 (39)	8 (35)		44 (27)	28 (17)	17* (13)	32 (19)	
E	—	—	—	—	—	37 (22)	24* (18)	42 (25)	
F	7 (30)	8 (35)	0 (0)	8 (35)	—	—	15* (11)	28 (17)	
G	—	—	—	—	—	—	—	18* (14)	
H	6 (26)	6 (26)	6 (26)	4 (17)	—	6 (26)	—		

The number of amino acid replacements in each pair of coding sequences are shown. The 23 amino acid signal peptides are compared in the lower left part and the 166 amino acid mature LeIFNs are compared in the upper right part of the table. The total number of differences between pairs is listed first, followed by the percentage difference (numbers in parentheses). Signal sequences for LeIFNs E and G are not known, thus there are no values for comparisons involving either of these sequences.

* Only the 133 C-terminal amino acids of LeIFN G can be deduced from the cDNA sequence, thus in pairwise comparisons involving LeIFN G only differences in these 133 amino acids are considered.

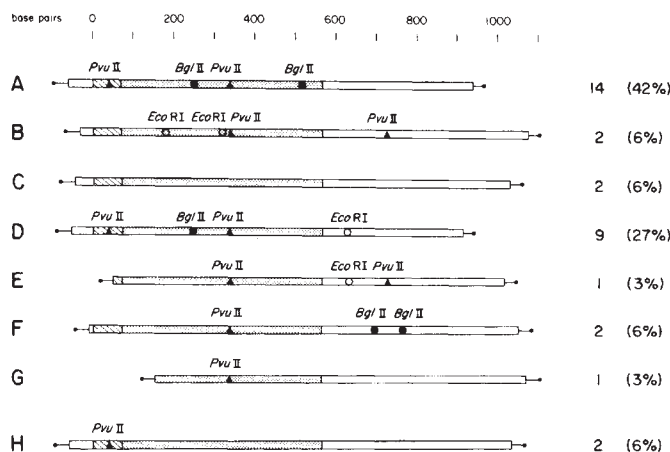


Fig. 2 Restriction endonuclease maps of the eight types of LeIFN cloned cDNAs (A-H). 33 LeIFN cDNA clones with *Pst*I cDNA inserts >700 base pairs were grouped into these eight classes. The hybrid plasmids were constructed by the dC:dG tailing method⁵. Therefore, the cDNA inserts could be excised using *Pst*I. The lines at the end of each cDNA insert represent the flanking homopolymeric dC:dG tails. The positions of *Pvu*II, *Eco*RI and *Bgl*II restriction sites are indicated. Shaded regions indicate the coding sequences of mature LeIFNs, cross-hatched regions indicate signal peptide coding sequences, and the open regions show 3' and 5' noncoding sequences. The numbers at the right are the total number of cDNA clones in each group, with the per cent in each group in parentheses.

A LeIFN pseudogene is transcribed

The coding region of the LeIFN E cDNA clone contains one nucleotide more than the other LeIFN cDNAs (Fig. 3). The predicted amino acid sequence from this nucleotide insertion onwards (amino acid residue 40 in LeIFN E) bears no resemblance to the other LeIFN protein sequences and results in several in-phase termination codons, the first of which would appear at amino acid position 60. Therefore, the amino acid sequence is shown in Fig. 4 as if this insertion had not occurred. Even with this adjustment, termination codons are encountered at positions 102 and 155—thus they are found in all three reading frames. As a cDNA was cloned that contains an additional nucleotide in the coding sequence for a LeIFN, an artefact may have been created during the process of reverse transcription of mRNA and sequence amplification in *E. coli*. However, the presence of the two additional termination codons could imply that LeIFN E is a copy of a pseudogene analogous to the α - and β -globin pseudogenes²⁷⁻²⁹. If so, this is the first reported evidence of a pseudogene being transcribed into RNA. The biological significance of the transcription and possible translation of this pseudogene has not been determined. The fact that only one cDNA clone of a LeIFN pseudogene was obtained in our extensive search for LeIFN cDNA clones suggests that the transcription of such genes may be very rare. This observation is further supported by our recent isolation from a human chromosomal DNA library of an additional LeIFN pseudogene with a DNA sequence distinct from the eight LeIFN cDNA clones described here (unpublished results).

LeIFN signal peptides

The signal sequences of human LeIFNs A, B, C, D, F and H possess features common to all eukaryotic secretory protein signal sequences. Each contains a long stretch of hydrophobic amino acid residues, perhaps important for association with, and traversal of, the rough endoplasmic reticulum membrane. The hydrophobic regions are flanked by charged and more polar amino acid residues. The signal sequences are rich in serine residues proximal to the prepeptide cleavage site, analogous to the signal sequences of rat proalbumin³⁰ and certain mouse

immunoglobulin chains³¹. Within the cell, removal of the signal peptides is presumably achieved by peptide cleavage between glycine and cysteine residues in all of these LeIFNs.

The signal sequences of many different proteins have been determined and compared³². No major sequence homologies have been detected; the most general feature may be a common secondary or tertiary structure (see ref. 33 for review). It is possible that the junction of distinct structural domains within the signal peptide is recognized by a processing enzyme or signal peptidase³⁴, resulting in production of the mature secreted protein and complete translocation into the lumen of the rough endoplasmic reticulum.

The signal sequences of the LeIFNs reported here are only ~70% homologous when compared with each other, except for types C and F, which are identical (Table 1). Only 11 of the 23 amino acids (43%) occur in invariant positions in all six prepeptides. The sequence of the human FIFN signal peptide^{7,18,20} is completely different from those of the human LeIFNs.

Mature leukocyte interferons

The mature LeIFNs are more closely related in their amino acid sequences (~80% homology) than are their corresponding signal peptides (see Table 1). A total of 79 out of 166 amino acids (48%) of the mature polypeptide are identical in all sequences examined (Fig. 4). If the LeIFN E pseudogene amino acid sequence is ignored, then 99 amino acids (60%) occupy identical positions. When the human FIFN sequence^{7,18,19,21} is compared with the LeIFN sequences, only 39 amino acids out of 166 (23%) appear in identical positions (Fig. 4). The overall distribution of amino acid differences along the LeIFN molecules seems to be random, with the possible exception of the region between amino acids 115 and 151. All the LeIFN cDNA clones (again excluding LeIFN E) code for the same amino acid in 31 out of 37 (84%) positions in this region. Residues 116-150 also display the greatest similarity to FIFN (17 out of 35 positions identical), which suggests that this region is of functional importance.

The changes in amino acid sequences between the LeIFNs are largely due to single nucleotide changes; there are several sites (that is, positions 37, 107 and 160) where nonconservative amino acid changes occur. Note that the nucleotide sequence of LeIFN B contains an insertion of a thymidine residue after nucleotide 362 (codon 98), which changes the translational reading frame and results in four distinct codon changes. After position 373 (codon 101) an adenosine residue has been deleted which restores the original reading frame:

	97					102
	Glu	Ala	Cys	Val	Ile	Gln
LeIFN A	GAA	GCC	TGT	GTG	ATA	CAG
	Glu	Val	Leu	Cys	Asp	Gln
LeIFN B	GAA	GTC	CTG	TGT	GAT	CAG

The shorter (~800 base pairs) of the two LeIFN H-type cDNA clones, which we have designated LeIFN H₁, has a deletion (cytidine, nucleotide number 545) which is followed six nucleotides later by a correcting insertion (guanosine, after position 551):

	158				162
	Leu	Gln	Lys	Arg	Leu
LeIFN H	TTG	CAA	AAA	AGA	TTA
	Leu	Lys	Lys	Gly	Leu
LeIFN H ₁	TTG	AAA	AAA	GGA	TTA

Except for this difference and a single base change in the 3' noncoding regions, the nucleotide sequences of LeIFN H and LeIFN H₁ cDNAs are identical. It is possible that these two cDNA clones may represent allelic forms of a single LeIFN gene found in the KG-1 cell line.

The location of cysteine residues is often highly conserved in proteins. All the LeIFNs sequence code for cysteines at positions 1 and 139 of the mature protein. All predicted LeIFNs

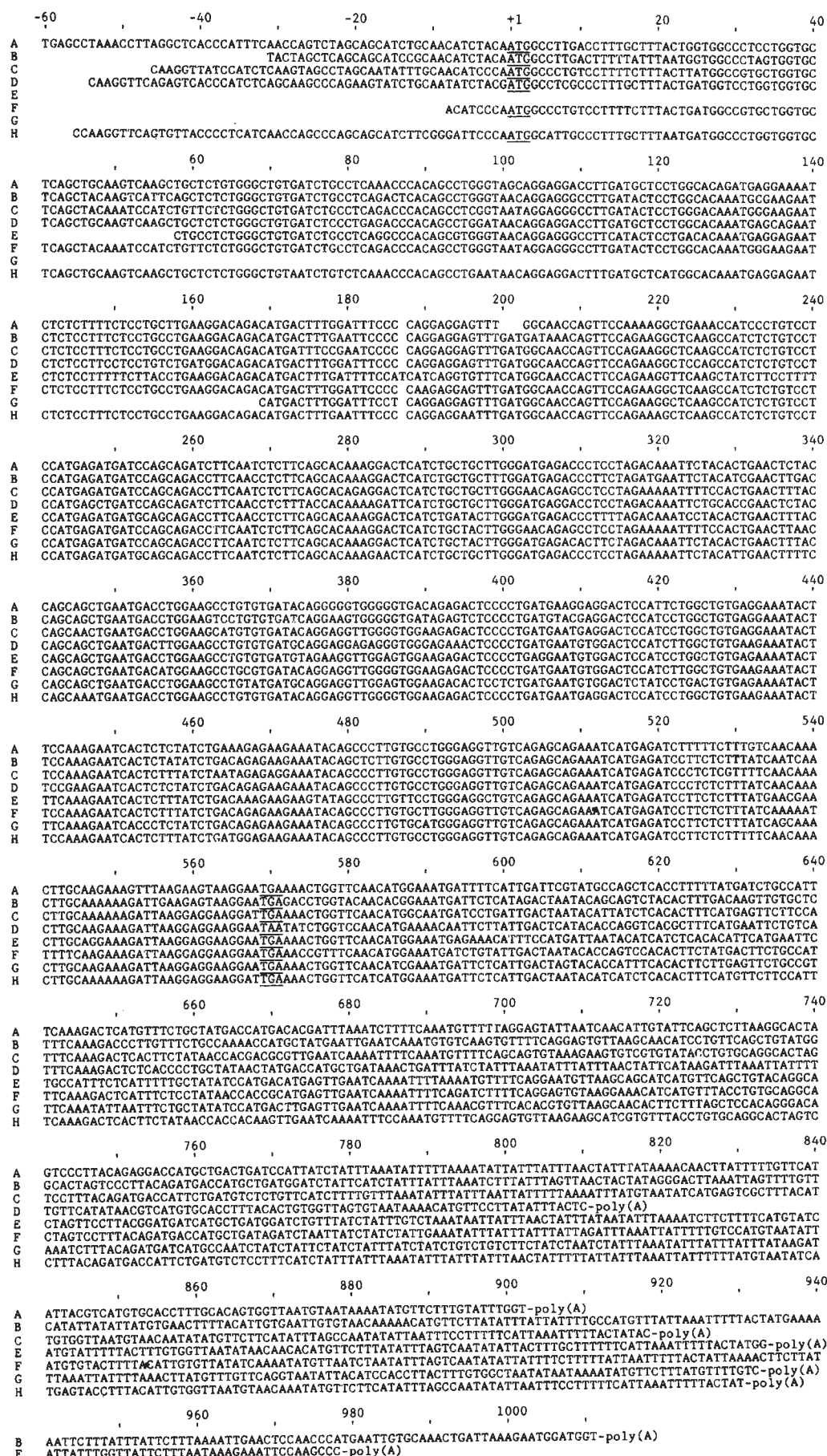


Fig. 3 Nucleotide sequences of the eight LeIFN cDNAs (A-H). DNA sequences of the *Pst*I inserts shown in Fig. 2 were determined by the Maxam-Gilbert technique²⁴ and by the dideoxy chain termination procedure after subcloning into the M13 vector mp7 (ref. 25). The ATG translational initiation codon and the termination triplet for each LeIFN are underlined. The gaps at position 187 were introduced to maximize homologies in the coding regions.

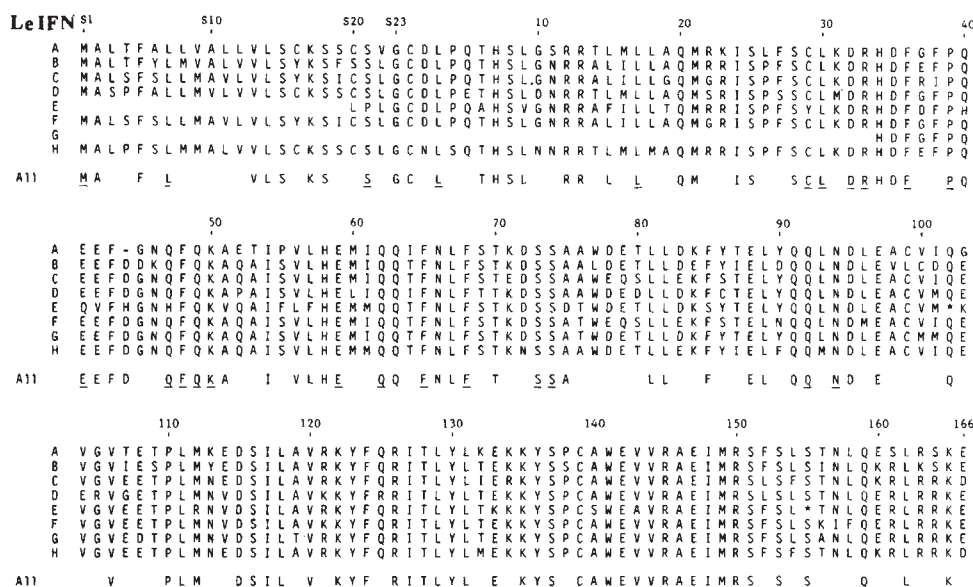


Fig. 4 Comparison of the eight LeIFN protein sequences (A-H) predicted from nucleotide sequences. The one-letter abbreviations recommended by the IUPAC-IUB Commission on Biochemical Nomenclature⁴⁶ are used: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine. The numbers refer to amino acid position (S refers to signal peptide). The dash in the 165 amino acid LeIFN A sequence at position 44 is introduced to align the LeIFN A sequence with the 166 amino acid sequences of the other LeIFNs. The LeIFN E sequence was determined by ignoring the extra nucleotide (position 187 of Fig. 3) in its coding region. Asterisks indicate in-phase termination codons. Amino acids common to all LeIFNs (excluding the pseudogene LeIFN E) are also shown. The underlined residues are amino acids which are also present in human FIFN^{7,18,19,21}.

except LeIFN E contain a cysteine at position 29, and all except LeIFN B (Cys 100) contain a cysteine at position 99. At least one intramolecular disulphide bridge has been predicted on the basis of loss of interferon activity on incubation with reducing agents³⁵. Allen and Fantes¹³ have shown that the N-terminal cysteine is probably involved in a disulphide bond. Furthermore, the presence of two disulphide bridges in the LeIFN A produced in *E. coli*² has been established by analysis of tryptic digests of the purified protein: Cys 1 is bonded to Cys 99 and Cys 29 is bonded to Cys 139 (ref. 47). The fact that cysteine residues are also predicted in human FIFN^{7,18,19,21} in the positions corresponding to amino acids 29 and 139 of LeIFN suggests that this disulphide bond also occurs in FIFN.

NH₂-terminal sequences of human LeIFN species isolated from lymphoblasts^{12,13} and leukocytes¹⁴ have been determined by protein microsequence analysis. The sequences determined by Zoon *et al.*¹² and Levy *et al.*¹⁴ both have NH₂-terminal serine residues. The sequences published by Allen and Fantes¹³ begin with cysteine residues. The seven full-length LeIFN cDNAs (A-F, H) all code for mature LeIFNs beginning with cysteine. Although it is possible that a LeIFN cDNA clone will be identified which encodes an N-terminal serine, it is more likely that the determination of serine arose artefactually through oxidation of cysteine during the initial protein microsequencing procedure.

Disregarding the discrepancies at amino acid position 1, the following comparisons can be made. The 32 NH₂-terminal amino acids of the lymphoblastoid IFN sequence of Zoon *et al.*¹² have the greatest homology with LeIFN F, with only position 26 (Pro in LeIFN F, Leu in lymphoblastoid IFN) differing between the two species. The 22 NH₂-terminal amino acids of LeIFN determined by Levy *et al.*¹⁴ show the most homology with LeIFN A. These differ only at position 11 (Asn in the Levy sequence compared with Ser for LeIFN A). Allen and Fantes¹³ have determined the sequences of tryptic and chymotryptic peptides of two forms of LeIFN: IFN- α A and IFN- α B¹³. IFN- α A comprises at least two different polypeptides of 165 amino acids with the same amino acid deletion at position 44 that is predicted by the LeIFN A cDNA sequence. 140 of the 142 amino acids which have been determined from the IFN- α A peptides correspond to the LeIFN A cDNA sequence presented

here; only Asp 84 and Pro 88 of IFN- α A are not predicted by the LeIFN A cDNA sequence. IFN- α B consists of at least three polypeptides of 166 amino acids each and the residues present at 161 positions have been determined¹³. Peptides of IFN- α B are most closely related to LeIFN D, with only Met 60, Thr 64 and Ala 114 not being predicted by the LeIFN D cDNA sequence. The 77 N-terminal amino acids of LeIFN F are found in the IFN- α B peptide sequences. However, there are 11 differences between these two interferon sequences in the C-terminal portion (residues 78-166) of the polypeptides. Amino acid sequences deduced from LeIFN B, C, G and H cDNAs do not seem to be represented in either IFN- α A or IFN- α B peptide sequences. These comparisons suggest the presence of additional LeIFN genes, cDNA copies of which are not present, or have not been identified, in our cDNA library. Whether some of the slight differences between LeIFN sequences described above could be due to allelic variation in the human population awaits a detailed genomic characterization of the LeIFN gene family from an individual.

Noncoding sequences of LeIFN

A limited amount of sequence information is available for the 5' noncoding regions of the LeIFN cDNAs (Fig. 3). Obvious homologies exist in the regions immediately preceding the translational initiation codon; LeIFNs A, B and D are similar in this region, as are LeIFNs C and F.

The 3' noncoding regions of the LeIFN cDNA clones vary in size from 242 (LeIFN D) to 441 (LeIFN B) base pairs, counting from the first nucleotide of the stop codon to the site of polyadenylation. Figure 5 shows an alignment of these sequences with gaps introduced to achieve maximum homology. These gaps may be sites of deletions or insertions occurring during the evolution of this multigene family³⁶. The overall homology between 3' noncoding regions is lower than that within the protein coding regions, although certain portions of the 3' noncoding region are relatively conserved. Both the reduced level of 3' noncoding homology and the substantial deletions in the sequence alignments resemble the situation found when comparing the analogous DNA sequences of members of the human β -like globin gene family³⁶. Such results suggest that the precise sequence of much of the 3' noncoding regions of

eukaryotic mRNAs is not essential for function. Experiments involving *in vitro* translation of mRNAs with large 3' deletions support this suggestion^{37,38}.

Efstratiadis *et al.*³⁶ have noted that short (2–8 base pairs) tandem repeats often surround the deletion sites in the β -like globin gene family 3' noncoding sequences. They suggested a mechanism of 'slippage' during DNA replication followed by scission of resulting single-stranded DNA loops as a possible cause of these deletion events during evolution. Such short tandem repeats also occur at many of the deletion points in the LeIFN cDNA sequences.

The hexanucleotide AATAAA precedes the site of polyadenylation in many eukaryotic cellular mRNAs by 15–25 bases³⁹. Although exceptions to the rule have been found in several RNA viruses⁴⁰, this sequence is thought to be involved in mRNA processing and/or polyadenylation. A variant of this sequence, AATTAAA, has recently been reported which precedes the poly(A) site in rat amylase⁴¹ and anglerfish somatostatin⁴² mRNAs. LeIFN A, D, F and G cDNAs contain the hexanucleotide AATAAA ending 20, 21, 14 and 21 nucleotides, respectively, from their polyadenylation sites. LeIFN B, C, E and H cDNAs do not contain this hexanucleotide. At the nucleotide position (nucleotides 347–352, Fig. 5) corresponding to AATAAA in the LeIFN A, D and G cDNAs, the sequence of the other cDNAs differ by one or two nucleotides. This sequence divergence may have led to the extension of the 3' noncoding region in those genes. While LeIFN F mRNA continues until an AATAAA is encountered 14 nucleotides before its poly(A) site, LeIFNs C, E and H possess the related hexanucleotide ATTAAA near their 3' termini (positions 399–404). LeIFN B also has the ATTAAA sequence at this position, but is not polyadenylated until another ATTAAA sequence is reached at positions 484–489. Note that the two cDNA clones most often isolated (LeIFN A and D) have the shortest 3' noncoding sequences and possess the canonical hexanucleotide at the same position.

Significance of the LeIFN multigene family

We have presented here the sequences of eight distinct human LeIFN cDNAs, seven of which probably code for functional interferon polypeptides. LeIFN amino acid sequence information^{12–14} suggests the presence of additional LeIFN structural genes. The genomic blots shown in Fig. 1 and the isolation of chromosomal LeIFN sequences¹⁵ also support the concept of a LeIFN multigene family comprising at least 8–10 distinct human LeIFN-related genes, although some of these could be pseudogenes similar to LeIFN E.

The cDNA clones LeIFN A and D occur relatively frequently in our cDNA library⁵, which was prepared using RNA from the virus-induced myeloblastoid cell line KG-1¹⁰. In contrast, a major LeIFN species found in the lymphoblastoid Namalwa cell line¹² most closely resembles the polypeptide encoded by LeIFN F cDNA—this supports the suggestion of Nagata *et al.*¹⁵ that different cell types might preferentially express different LeIFN genes. Preferential expression of different IFNs might also occur in the same cell line in response to different viruses.

These multiple LeIFNs probably show distinct functional properties. Streuli *et al.*⁶ have shown that two different LeIFNs (IFN- α 1 and IFN- α 2) have striking differences in their antiviral activities on cells of different species. We have recently obtained expression in *E. coli* of the mature LeIFN polypeptides encoded by LeIFN A, B, C, D and F cDNAs (unpublished results) and all five show different cross-species specificities. The availability of these cloned LeIFNs should allow the assignment of the various activities characteristic of mixed leukocyte interferon preparations² to individual LeIFN species. A detailed understanding of the correlation between LeIFN structure and activity may lead to the design of new interferons highly active against particular diseases.

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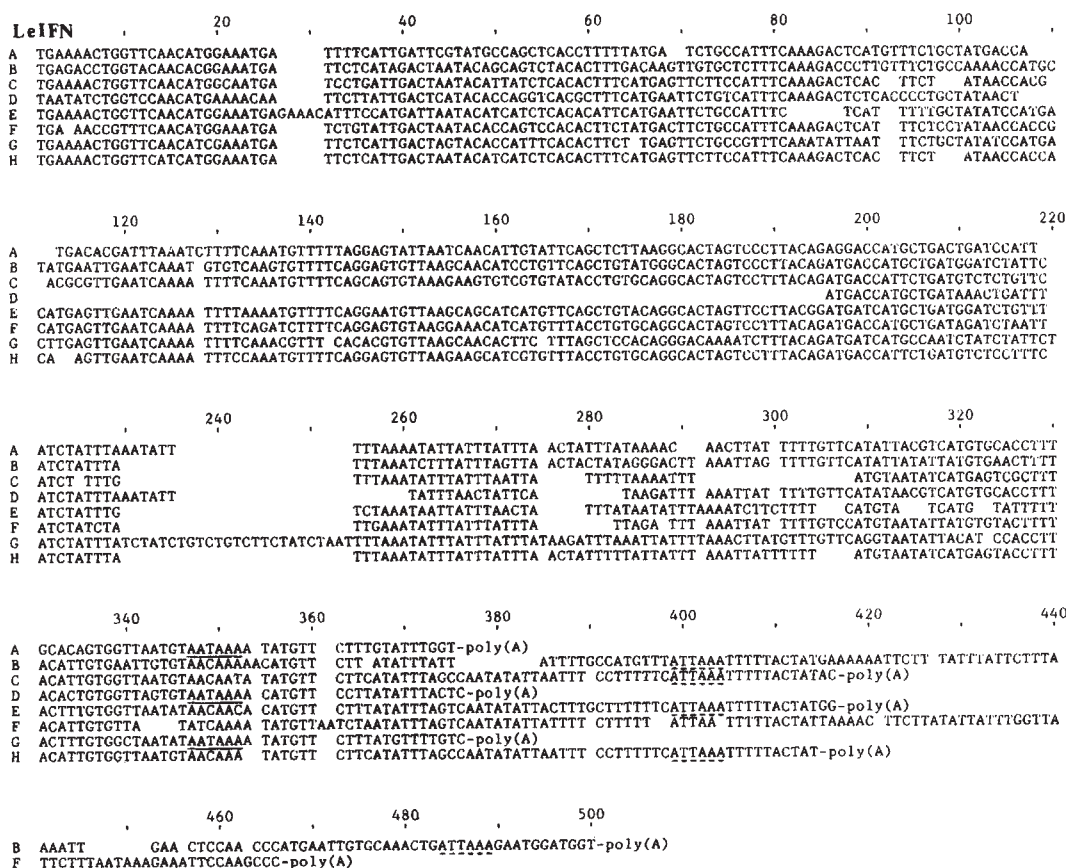


Fig. 5 Alignment of the 3' noncoding sequences of eight human LeIFN cDNAs. The alignment of sequences between the termination codon and the poly(A) addition site was made using considerations described elsewhere³⁶. Blank areas indicate gaps introduced to achieve consistent alignments. The sequence AATAAA common to most eukaryotic polyadenylated mRNAs³⁹ is underlined. The related sequence ATTAAA, which may also serve as a polyadenylation signal, is indicated by dashed lines.

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Homology and concerted evolution at the $\alpha 1$ and $\alpha 2$ loci of human α -globin

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The identical structure of two racially distinct $\alpha 1$ -globin alleles and the high degree of homology between the $\alpha 1$ - and $\alpha 2$ -globin loci indicate that mechanisms exist for suppression of allelic polymorphisms and for exchange of genetic information within the α -globin gene complex.

THE human α -globin polypeptide chains are encoded at two closely linked loci^{1,2} on chromosome 16 (ref. 3). Genomic DNA containing both of these genes has been cloned and mapped⁴. The loci are 3.2 kilobases apart and are oriented in the same transcriptional direction. The more 5' or leftward α -globin gene has been designated as $\alpha 2$ and the more 3' or rightward α -globin gene as $\alpha 1$. The α -globin proteins encoded at these loci have remained identical⁵ even though the duplication of the α -globin gene probably occurred before the mammalian divergence 85 Myr ago and possibly before the bird-mammal divergence a minimum of 300 Myr ago⁶. As gradual accumulation of mutations within these two genes would have caused divergence in the primary structure of the coded proteins over this time period, a mechanism for correcting one gene against another has been postulated⁷. The parallel or coincidental evolution of two genes (concerted evolution⁶) may occur by homologous but unequal cross-over between the two mutually correcting genes. Comparison of α -globin protein structure in primates indicates that this interaction between the two α -globin loci occurs at a high frequency⁶. Comparisons of the structure of the two γ -globin loci (γ^A and γ^G) support the concept of concerted evolution and suggest mechanisms for its implementation^{8,9}. Heteroduplex analysis⁴ and partial sequencing¹⁰ of the DNA within the human α -globin cluster have identified three homologous units that may align the $\alpha 1$ - and $\alpha 2$ -globin genes on sister chromatids, facilitating unequal cross-over between these two loci. To expand on these findings, we have sequenced the $\alpha 1$ -globin genes from two individuals of different races and compared these alleles with each other and with the structure of the $\alpha 2$ -globin gene¹¹. The two $\alpha 1$ -globin alleles are identical, whereas the $\alpha 1$ and $\alpha 2$ loci have diverged in a defined manner. These observations are consistent with frequent exchanges of DNA within the α -globin gene cluster.

Primary structure of two $\alpha 1$ -globin alleles is identical

The $\alpha 1$ -globin genes cloned from the DNA of an Asian (pGL $\alpha 1$) and a Caucasian (pRB $\alpha 1$) were sequenced in parallel. The sequencing scheme used is shown in Fig. 1 and the regions

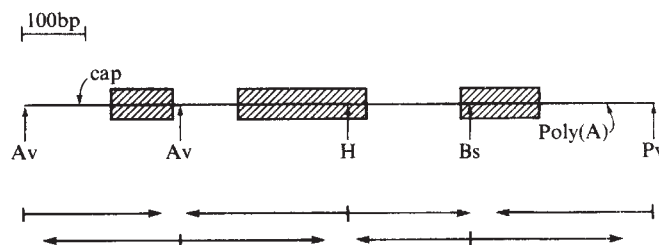


Fig. 1 Strategy for sequencing the $\alpha 1$ -globin genes. Vertical arrows denote restriction enzyme cleavage sites; *AvaI* (Av), *HindIII* (H), *BstE2* (Bs), *PvuII* (Pv). The positions of the capping and poly(A) addition sites as determined from the mRNA^{28,29} are shown. DNA was cut at each of the indicated restriction sites and either 5'-labelled with polynucleotide kinase (BRL) and [γ -³²P]ATP (Amersham, 3,000 Ci mmol⁻¹) after dephosphorylation of the DNA with bacterial alkaline phosphatase (BRL), or 3'-labelled with *Escherichia coli* DNA polymerase I, Klenow fragment (BRL) and the appropriate [α -³²P]dNTP (3,000 Ci mmol⁻¹). These labelled fragments were purified on 5% acrylamide gels and either strand separated³⁰ or secondarily cut by another restriction endonuclease. Gel-purified end-labelled pieces were sequenced by the method of Maxam and Gilbert³⁰. The horizontal arrows are proportional to the distance each fragment was read. All fragments were sequenced twice and in most cases on both strands. bp, Base pairs.

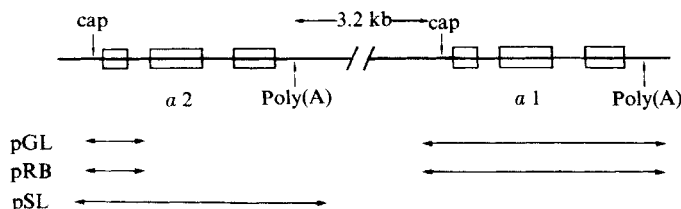


Fig. 2 Sequencing summary. The complete sequence of the $\alpha 2$ -globin allele *pSL* $\alpha 2$ has been reported¹¹. *pGL* $\alpha 1$ and $\alpha 2$ are *pBR* 322 subclones containing respectively the $\alpha 1$ - and $\alpha 2$ -globin genes from a single chromosome (M. Goossens, unpublished data). These were subcloned from a Charon 4A recombinant containing a 14.5-kilobase (kb) *Bam*HI fragment that includes both α -globin genes¹. The DNA originated from the leukocytes of a Chinese subject. *pRB* $\alpha 1$ and $\alpha 2$ are *pBR* 322 subclones containing the $\alpha 1$ - and $\alpha 2$ -globin genes from a single chromosome (γ H α G2)⁴ of a Caucasian patient and were provided by T. Maniatis. The plasmid subclones used for sequencing were grown in 1.5-l batches, amplified at A_{640} 0.7 with chloramphenicol ($150 \mu\text{g ml}^{-1}$), collected 18 h later and purified from a clarified lysate by CsCl isopycnic centrifugation. The extent to which each of these genes was sequenced is indicated by the horizontal arrows (see text for details). The $\alpha 1$ - and $\alpha 2$ alleles were sequenced in parallel to enhance their comparison and to minimize misreadings. Each gene was labelled at the same restriction site, chemical reactions were done simultaneously and, in most instances, the modified and cleaved fragments were electrophoresed side by side on the same gels.

compared in each clone are summarized in Fig. 2. No differences are present in the primary sequence as determined by a direct visual comparison of the adjacent sets of sequencing lanes. Likewise, the sequences of the 230-base pair *Ava*I fragments (Figs 1, 2) encompassing the 5' end of three $\alpha 2$ -globin genes (*pSL* $\alpha 2$, *pGL* $\alpha 2$ and *pRB* $\alpha 2$) are identical and limited sequencing of the 3' end of a fourth $\alpha 2$ -globin allele (Chinese) from the *Bst*E-2 site at AA104 (Fig. 1) to the polyadenylation [poly(A)] site (G. Temple, personal communication) is identical with our reported $\alpha 2$ sequence for a Caucasian (*pSL* $\alpha 2$)¹¹ (see Fig. 2). This degree of homology was unexpected as Jeffreys has estimated that 1% of the bases in the human β -globin cluster are sites of polymorphism¹². However, this 1% estimate is based on the identification of three restriction site polymorphisms located in the large intervening sequence of the γ and δ genes. Subsequent sequencing of these genes has revealed that an unusually high rate of sequence divergence exists in this region¹³ and that the two polymorphisms noted in the γ -globin genes are probably due to a single initial mutation in one of the γ -globin genes with subsequent transfer of this mutation to the other γ -globin gene⁹. Therefore a reliable overall rate of sequence divergence in the β -globin complex must await comparative sequencing along the entire length of the genes in question.

3' border of homology between the α -globin loci is near the UAA termination codon

Comparison of the α -globin gene sequence at the $\alpha 1$ and $\alpha 2$ loci¹¹ reveals no differences in the 5'-flanking region, the 5'-nontranslated segment, the first exon, or the first intervening sequence (IVS). Exons 2 and 3 each contain one silent third-base substitution. Codon 54 (Gln) is encoded by CAG in $\alpha 1$ and by CAA in $\alpha 2$, and codon 123 (Ala) by GCC in $\alpha 1$ and GCT in $\alpha 2$.

The IVS2 of the $\alpha 1$ -globin gene is nine bases longer and differs by three bases from the same region of the $\alpha 2$ -globin gene (*pSL* $\alpha 2$). Position 55 of IVS2 is T in $\alpha 2$ -globin and G in $\alpha 1$ -globin. The remaining two base changes and all nine inserted/deleted bases are clustered toward the 3' end of this intron. This finding is consistent with the previous comparison of the *pRB* $\alpha 1$ and *pRB* $\alpha 2$ restriction maps which identified a

seven-base insertion/deletion in the same region of IVS2 (ref. 4). Figure 3 shows an alignment of this region that maximizes homology and assumes that the nine bases were deleted as three separate events. The seven-base deletion is flanked by a four-base direct repeat. Deletions in prokaryotic models¹⁴ as well as a number of sequence deletion within the β -globin gene cluster¹³ are similarly flanked by direct repeats in the precursor molecule. Whether the underlined four-base repeat (GGCC, Fig. 3) results in deletion by strand mismatch during DNA replication as suggested by these authors remains speculative, as the current data do not eliminate the possibility that the differences were created by insertions or insertion/deletion combinations.

Heteroduplex mapping studies define several large repeated homologous regions encompassing the $\alpha 1$ - and $\alpha 2$ -globin genes⁴ (Fig. 4a). Proudfoot and Maniatis¹⁰ have sequenced the actual borders of some of these segments. The most leftward homology box begins just 3' to the poly(A) addition site of the pseudo- $\alpha 1$ - and $\alpha 2$ -globin genes. This area includes a large intergenic region of homology between pseudo- α and $\alpha 2$ and $\alpha 2$ and $\alpha 1$ (the X box in ref. 11) (Fig. 4a). Based on the assumption that the 3'-noncoding region of $\alpha 1$ - and $\alpha 2$ -globin mRNA was identical (see below), the poly(A) addition site of these two genes was thought to define the 3' border of a second set of homology regions (the Z box) which encompasses the two α -globin genes and extends 1.5 kilobases in the 5' direction. Our sequence comparison of the 3'-noncoding region of the $\alpha 1$ and $\alpha 2$ genes (Fig. 4b) defines 18 base differences plus a single-base insertion or deletion, making this region only 84% homologous. Thus we would reassign the 3' border of the Z box (the border of α -globin gene recombination—see below) to a position 14 bases 3' to the UAA termination codon. The assumption that the 3'-noncoding regions of both α -globin genes were identical was based on the determination of a unique sequence for this region from reticulocyte mRNA¹⁵. That sequence is almost identical to the gene sequence of the $\alpha 2$ -globin locus, whereas it differs from the $\alpha 1$ -globin gene at 18 bases and one insertion or deletion. Although a definite sequence assignment could not be made at seven positions, only three of these correlate with the base differences between the $\alpha 1$ - and $\alpha 2$ -globin genes. This disparity between the unique sequence of reticulocyte α -globin mRNA and the above genomic sequence data may be reconciled by suggesting that the mRNA transcribed from the $\alpha 2$ -globin locus predominates in circulating reticulocytes due to unequal production or degradation¹⁶.

The sequence divergence between $\alpha 1$ - and $\alpha 2$ -globin genes which begins 3' to the UAA terminator codon and extends into the 3'-flanking region is interrupted by a 50-base pair homologous stretch bridging the poly(A) site and the AAUAAA hexanucleotide (poly(A) signal)¹⁷ (Figs 4b, 5). This region (with the exception of the conserved hexanucleotide AAUAAA) is highly divergent in eukaryotic mRNA in general, and in β -globin can be deleted or inactivated without adversely affecting *in vitro* translational activity¹⁸. Among β -globin and β -like globin genes homology in this region is significant only for the hexanucleotide AAUAAA, its immediately surrounding bases and the sequences adjacent to the poly(A) addition site¹³. Thus the observed maintenance of perfect homology in a segment of

GGC_GCGG_CTGGGCC_____GCACT	$\alpha 2$
GGCTGCGGGCCTGGGCC_____CCACT	Possible transitional sequence
GGCTGCGGGCCTGGGCCCTCGGCCCACT	$\alpha 1$

Fig. 3 Deletion sites in the 3' end of IVS2. This sequence begins at base 101 of IVS2 and extends through the divergent region to a position 19 base pairs from the junction of IVS2 with exon 3. The sequence of the $\alpha 1$ -globin gene, the $\alpha 2$ -globin gene¹¹ and a possible intermediate sequence are aligned for maximal homology. The open spaces in the $\alpha 2$ -globin gene and the intermediate sequence represent deletions in this region relative to the $\alpha 1$ -globin gene. The dot indicates the position of a base change. The underlined tetranucleotide GGCC is discussed in the text.

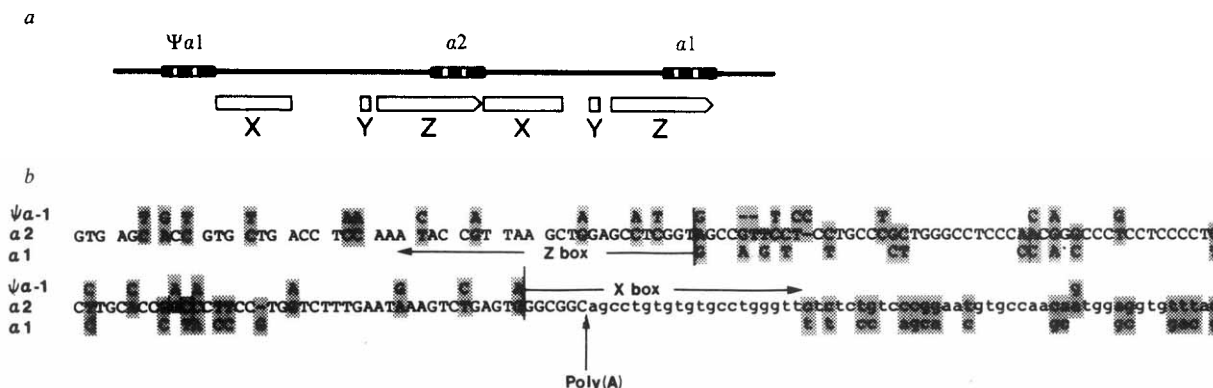


Fig. 4 Homologous regions within the segment of α -globin gene cluster containing the $\psi a1$ -, $a2$ - and $a1$ -globin genes. The rectangular blocks represent the respective genes and the open spaces within these blocks represent the introns. Regions that show identity by heteroduplex analysis⁴ or partial sequence determination¹⁰ are given the same letters (X, Y or Z). This figure is adapted from ref. 10 by permission of the author (N.J.P.). **b**, Comparison of the 3' ends of the $a1$ -globin, $a2$ -globin and $\psi a1$ -globin genes¹⁰. The sequence begins at the codon for AA132 of the functional α -globin genes and extends through the TAA ochre codon, the 3'-nontranslated region, and into the 3'-flanking region (lower-case letters). Base differences between the $a1$ -globin gene and the $a2$ -globin gene or $\psi a1$ -globin gene¹⁰ are indicated by the shaded boxes. Deletions are indicated by a hyphen. The region of homology encompassing the $a1$ - and $a2$ -globin genes (Z box) has its 3' border approximately 14 bases 3' to the TAA termination codon and the region of homology 3' to the $\psi a1$ - and $a2$ -globin genes (X box) has its 5' border six bases 5' to the poly (A) addition site.

this region is probably not entirely explained by functional constraints and could instead be due to a localized transfer of DNA between the two α -globin loci (see below).

Evolution of the α -globin genes

The $a1$ - and $a2$ -globin genes may exchange DNA by interaction between malaligned chromosomes. Such interactions may be facilitated by and result in large homologous regions within the α -globin complex (refs 4, 10; see also Fig. 4a). The degree of divergence within these regions would be proportional to the length of time since this exchange occurred. Divergence outside this unit (3'-untranslated and 3'-flanking area of the α -globin genes) might be proportional to some earlier event such as the original α -globin gene reduplication or a subsequent correction. The sequence divergence between the two α -globin genes from the 5'-flanking area to the UAA terminator is 8 bases out of 773 or 1.1% (each postulated insertion or deletion event is given the value of one base change). We assume that the changes within IVS2 are (phenotypically) silent, based on the finding in recently diverged gene pairs that the rate of accumulation of silent changes in coding regions approximately equals that in intervening sequences¹⁹. The 1.1% silent divergence can be equated to divergence time. For recent evolutionary events in the globins (less than 85 Myr) Efstratiadis *et al.*¹³ have calculated a unit evolutionary period (UEP) of approximately 1.3 Myr (1% silent sequence divergence per 1.3 Myr) based on the method of Perler *et al.*¹⁹. Using this constant, the divergence time or time

since the last correction between the $a1$ - and $a2$ -globin genes in this homologous region is 1.4 Myr. Similarly, Zimmer *et al.* have calculated that the last correction of the α -globin genes occurred 0.7 Myr ago⁶, based on amino acid differences in several primate species. The divergence in the 3'-noncoding region and 3'-flanking regions is 17.2% (19/110) and 27.8% (17/61), respectively. If the denominators are revised to exclude the 50-base pair homologous region that encompasses the poly(A) addition site, the divergence rates become 29% (19/66) and 42% (17/40) and the estimates of the divergence time become 40.3 and 54.6 Myr, respectively. As duplication of the α -globin gene has been estimated to have occurred before the bird-mammal evolutionary divergence (~300 Myr ago)⁶, the α -globin genes apparently have corrected against each other in the 3'-untranslated and flanking regions at least once and probably numerous times since the original duplication. The observation that the α -globin gene duplication unit contains segments with different levels of homology is not unique as a similar situation has been demonstrated in the γ -globin gene family⁹. It is assumed that the regions with higher homology have undergone more recent genetic exchange.

Evidence cited here and by others^{4,10} supports the concept of recombination and exchange of DNA between defined portions of the two α -globin loci. Mapping of the α -globin genes in patients with α -thalassaemia^{20,21} and confirmatory observations *in vitro* have demonstrated that the α -globin genes can recombine by unequal cross-over at a variety of sites (ref. 4, and

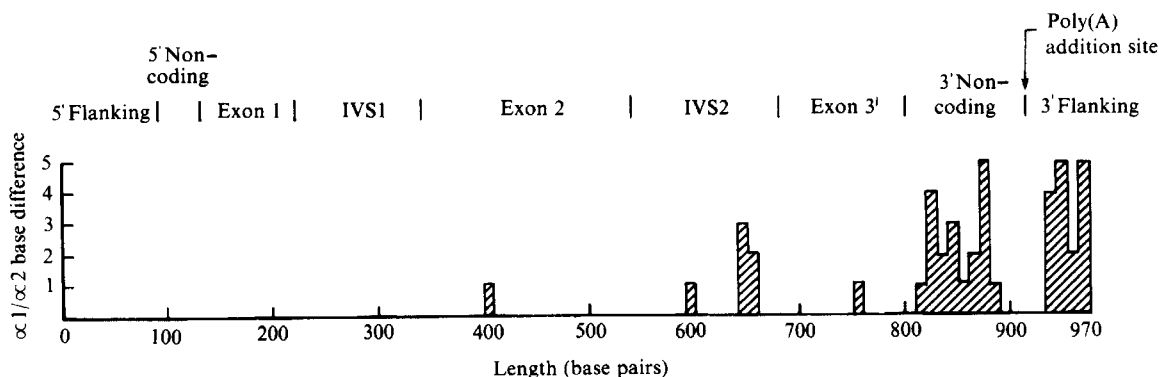


Fig. 5 Distribution of differences in the primary structure of the $\alpha 1$ - and $\alpha 2$ -globin genes. The width of each bar covers a 10-base pair segment and the height is proportional to the number of base differences within that segment. The analysis covers the α -globin genes from 88 base pairs 5' to the cap site and extends through the genes to 61 base pairs 3' to the poly (A) addition site. Each deleted base or group of bases was assigned a value of one base difference (see text).

M. Goossens, unpublished data). One outcome of such a strand exchange is a chromosome with three α loci^{22,31} and a chromosome with one α locus²³. Such interactions are made possible by base pairing between two malaligned DNA strands mediated by the repeated homology units encompassing the adjacent loci. The size of these homology units may be determined by the extent of the most recent strand exchange and this in turn would determine the target size and frequency of subsequent interactions. A succession of such strand exchanges of varying lengths would yield adjacent regions that vary in degree of homology. The variation in homology observed on either side of the $\alpha 1$ - and $\alpha 2$ -globin UAA termination codons may be explained by this mechanism. Although a similar mechanism has probably maintained the homology unit common to the two γ -globin genes⁹, this sort of exchange has not occurred at a comparable rate between the closely linked β - and δ -globin genes¹³. The δ -globin gene in all primates studied is either unexpressed or expressed to a very limited extent²⁴. If limited δ -globin gene expression is due to an abnormality in its structure, then an unequal cross-over between the δ -globin and β -globin genes, such as haemoglobin Lepore, might severely diminish the β -globin gene expression leading to a β -thalassaemia phenotype. As it is deleterious, such an exchange would probably be selected against in most populations.

Our comparative sequencing of the $\alpha 1$ -globin genes from an Asian and a Caucasian shows that the sequences of these two $\alpha 1$ alleles are identical. Assuming a UEP of 1.3 Myr (see above) and a target size of 900 base pairs (the region sequenced in both $\alpha 1$ -globin genes), one base difference is expected between the α -globin alleles every 140,000 yr. (This is a minimal estimate because the UEP of 1.3 Myr is for silent changes.) From the

accumulated globin sequencing data available, it seems unlikely that the sequence of each α -globin gene has been so stringently maintained entirely by selective pressure and hence the observed suppression of polymorphism may be the result of a genetic recombination such as that postulated to occur between the γ -globin genes⁹. A mechanism for such structural correction of one allele by another (gene conversion) has been reviewed by Radding²⁵. Homologous interaction between the corresponding loci of two aligned chromosomes which contain distinct alleles can result in a non-reciprocal exchange, with the loss of one allele and its replacement by the other. Such non-reciprocal sister chromatid exchange is observed in yeast²⁶ and *Drosophila*²⁷. As racial divergence probably paralleled population segregation, genetic interaction would not be expected to occur at high frequency across racial lines. Hence the finding of an identical α -globin gene sequence in Asians and Caucasians implies that these two races probably diverged after the establishment in a common precursor population of the most recent conversion at the $\alpha 1$ -globin locus.

Mechanisms of genetic exchange cited above can be constructed to account for the observed patterns of homology within the α -globin gene region. The large repeated regions within this gene cluster probably facilitate such interaction. However, it is unclear whether any selective advantage is gained by maintenance of homology within a duplicated gene locus. How the product of these interactions becomes predominant within a population remains to be defined.

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Homologous pairing can occur before DNA strand separation in general genetic recombination

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In the presence of ATP and Mg²⁺, purified Escherichia coli recA protein promotes the formation of joint molecules between closed circular duplex DNA and homologous circular single-stranded DNA carrying a short annealed fragment. The presence of this fragment is essential for pairing between molecules. In similar conditions recA protein is unable to act as a helicase and does not cause strand separation of the fragment from the single-stranded circle. Thus, homologous pairing between DNA molecules can take place without prior unwinding of a free end.

A GENETIC approach to the study of general genetic recombination has been to accumulate data derived from meiotic divisions in fungi and to develop models to illustrate how chromosomal DNA molecules might be cut, paired, joined and

matured into recombinant progeny^{1–5}. The models proposed by Holliday¹ and more recently by Meselson and Radding² have been used successfully to provide a coherent explanation for extensive data on recombination in fungi^{6,7}—the early steps of

Table 1 Detection of joint molecules by the retention of form I DNA on nitrocellulose filters, after reaction with substrate DNA consisting of Φ X174 viral DNA with annealed fragment

DNA	Proteins	% Duplex DNA retained
(1) Φ X174 substrate + form I Φ X174	—	7.7
Same	recA	19
Same	recA + SSB	37
(2) Φ X174 substrate + form I plasmid	—	7.7
Same	recA	3.0
Same	recA + SSB	5.6
(3) Single-strand Φ X174 + form I Φ X174	—	6.0
Same	recA	6.0
Same	recA + SSB	9.4
(4) Single-strand Φ X174 + form I plasmid	—	10
Same	recA	5.6
Same	recA + SSB	6.5
(5) Form I Φ X174 only	recA + SSB	8.3
Form I plasmid only	recA + SSB	8.0

The assay was essentially that described by Cunningham *et al.*²⁶. Reaction mixtures of 30 μ l contained 20 mM Tris-HCl, pH 7.5, 25 mM MgCl₂, 2 mM dithiothreitol, 100 μ g ml⁻¹ bovine serum albumin, 2 mM ATP, 1.7 nmol substrate or single-stranded Φ X174 DNA, 0.2 nmol ³²P-labelled form I Φ X174 or ³H-labelled form I plasmid pBR322 DNA, 40 μ g recA protein and 2 μ g SSB protein where indicated. After incubation at 37 °C for 30 min, reactions were terminated by the addition of 2 μ l of 0.25 M EDTA and proteins removed from the DNA by the addition of SDS to 1%. Water was added to bring the volume to 200 μ l, and 50 μ l of the reaction mixtures were spotted directly onto nitrocellulose filters (Schleicher and Schuell, BA 85) to measure total counts. The remainder of the reaction mixtures was added to 3 ml of iced 1.5 M NaCl, 0.15 M sodium citrate, pH 7.0, and filtered through nitrocellulose filters. These were then counted for radioactivity and the percentage of joint molecules calculated by determining the percentage of input form I DNA that was retained on the filter due to its hydrogen bonding to substrate DNA. Radioactivity due to the substrate DNA was determined in control experiments and subtracted before calculation of the percentage duplex DNA retained. In experiment 1, the difference of 29% in duplex DNA retained after incubation with recA and SSB proteins was subject to a statistical error of 3.3% (95% confidence limits). The substrate for this assay was prepared by digesting Φ X174 form I ³H-labelled DNA with *Hpa*I restriction enzyme (BRL) and separating the products on a 5% polyacrylamide slab gel. The smallest fragment (392 base pairs) was eluted from the gel, concentrated and denatured by heating, followed by rapid cooling. The fragments were then annealed with the (+) strands of Φ X174 ³²P-labelled DNA. Excess fragments not bound to the (+) strands were removed by neutral sucrose centrifugation. To measure the retention of intact circular DNA on filters that also retain the lightly labelled substrate DNA, highly labelled ³²P form I Φ X174 or ³H form I plasmid pBR322 DNA was used.

the latter model^{5,8} are shown in Fig. 1A. Recombination enzymes from fungi have been studied to a limited extent⁹; however, the cloning of bacterial recombination genes has facilitated the purification of their protein products and opened the way to an enzymological approach to the study of homologous pairing.

In *Escherichia coli*, recombination is controlled by *recA*¹⁰, the structural gene^{11,12} for a single-stranded (ss) DNA-binding protein of 38,000 molecular weight (MW)¹³⁻¹⁸ with ssDNA-dependent ATPase activity^{19,20}. Purified *recA* protein promotes homologous pairing between DNA molecules of various structures. Three situations have been studied: (1) in catalytic amounts, *recA* protein promotes hydrogen bonding between complementary single strands²¹; (2) in stoichiometric amounts, sufficient to cover all the ssDNA present, *recA* protein promotes hydrogen bonding between ssDNA fragments and homologous duplex DNA²²⁻²⁴. The product of the reaction is a D-loop, which contains a heteroduplex region with a single-strand fragment

paired to one strand of the duplex²⁵ (Fig. 1A, III). Pairing may be facilitated as *recA* protein reduces the winding number of duplex DNA²⁴; (3) *recA* protein also promotes homologous pairing between two duplex DNA molecules if one of them contains one or more short single-stranded regions^{26,27}. The latter reaction is of particular interest because genetic recombination in living cells usually involves duplex DNA molecules. Furthermore, because post-replication gaps in UV-ray-irradiated bacteria²⁸ are known to initiate sister exchanges with an efficiency approaching unity²⁹, it is likely that duplex DNA containing single-strand gaps (gapped DNA) is a natural substrate for recombination enzymes.

This important concept is supported by other results obtained with a gapped substrate. In the presence of ATP, *recA* protein recognizes gapped DNA and forms DNA-protein complexes with a high ATPase activity³⁰. If ATP is replaced by the non-hydrolysable analogue γ -S-ATP, this protein binds tightly to duplex DNA containing single-stranded regions, to form thick rod-like structures easily seen by electron microscopy. These rods are visible on gapped but not intact duplex DNA. Because homologous pairing requires ATP hydrolysis^{26,27}, we have suggested that rod-like structures formed transiently in the presence of ATP may participate in pairing between duplex molecules and the recognition of homology³⁰.

Two duplex molecules paired in a region of homology are sometimes referred to as joint molecules. The pairing of gapped DNA with intact homologous duplex DNA by *recA* protein results in joint molecules that are stable when deproteinized by SDS and proteinase K, but unstable in alkali^{26,27}. The paired molecules are linked by noncovalent bonds which show a thermal stability approaching that of duplex DNA²⁶. However, little is known about the structure in the pairing region of these joint molecules. Most published models for genetic recombination between two duplex DNA molecules assume that one duplex is

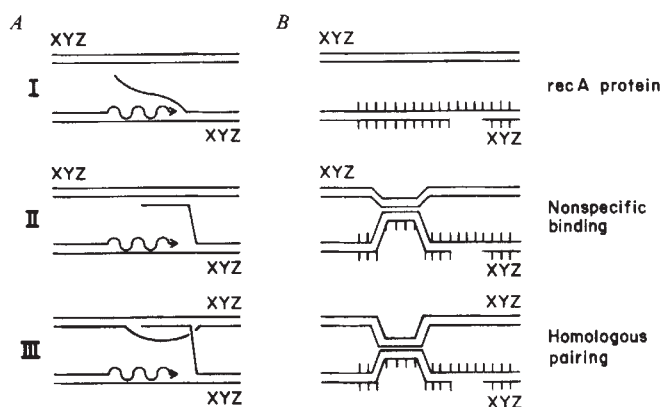


Fig. 1 Two models for the early steps in general genetic recombination. **A**, The initial steps from the model proposed by Meselson and Radding^{5,8}: I, displacement of a single strand by the replication of remaining strand. The wavy line denotes new synthesis; II, search for homology to single-stranded end; III, uptake of the free end of the displaced strand by a homologous double-stranded molecule to produce a three-stranded structure containing a heteroduplex called a D-loop^{22,25}. **B**, The initial steps of a model involving four-strand homologous pairing before strand separation: I, *recA* protein binds cooperatively to ssDNA at the site of the gap and loads the adjoining duplex; II, the loaded DNA binds nonspecifically and searches for homology in the intact duplex at the expense of ATP. The search may involve one molecule moving over the other or repeated contacts; III, formation of homologous contacts allows hydrogen bonding between the DNA molecules. The site of initial pairing may or may not include the position of the gap. The joint molecules are held by complementary hydrogen bonding, even after the removal of *recA* and SSB proteins. The subsequent steps which, in both models, include cutting, branch migration, isomerization and mismatch correction, are not shown here.

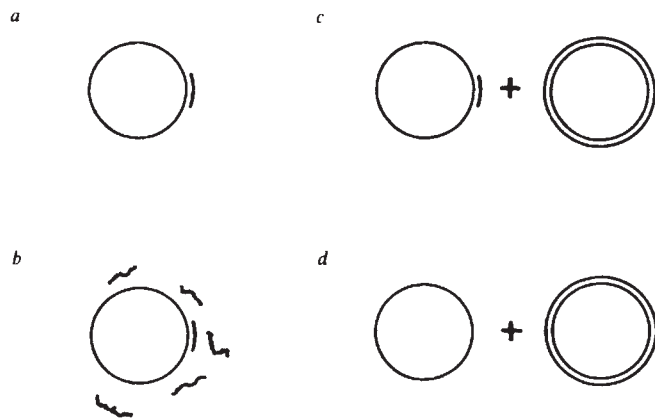


Fig. 2 DNA substrates used in the experiments. *a*, Single-stranded Φ X174 viral (+) DNA with a short annealed fragment (—); *b*, the same as *a* but with addition of sonicated (+) DNA fragments; *c*, same as *a* but with intact form I Φ X174 or plasmid pBR322 duplex circular DNA; *d*, single-stranded Φ X174 DNA with intact form I Φ X174 or plasmid duplex circular DNA.

cut or gapped, and the single strand with a free end is partially unwound, as shown in Fig. 1A, I (ref. 5). The unwound strand is thought to act as a template in the search for complementary sequences in the second duplex. If homologous contacts are made, the free end may wind into the intact molecule to form a heteroduplex, the remaining strand of the second duplex being displaced to form a D-loop (Fig. 1A, III).

However, the results of several experiments cannot be easily accounted for by a model for pairing between two duplexes which requires the initial unwinding of a free end: (1) the introduction of interstrand cross-links at both ends of the single-stranded region impairs strand separation but has no effect on the yield of joint molecules²⁷; (2) electron microscopic observations of joint molecules indicate that the joint is often distant from the single-stranded gap²⁶; (3) in addition, the experiments described here show that joint molecules can be formed by recA protein although helicase activity (unwinding and strand separation) could not be detected.

To account for these observations, we present here a working hypothesis for the initial step in homologous pairing between duplex molecules which is an alternative to D-loop formation. Unlike the Meselson–Radding model, it does not require new DNA synthesis and could therefore function in post-replication repair where replication is blocked by damaged bases^{28,29}.

RecA protein does not act as a helicase

Several proteins capable of catalysing the unwinding of duplex molecules have been characterized: helicases I, II, III and rep protein promote the unwinding of partially double-stranded (ds) DNA in ATP-dependent reactions^{31–35}. Incubation of these enzymes with a single-stranded substrate containing a short DNA duplex resulted in the separation of the strands of duplex DNA. This required the presence of single-stranded binding protein (SSB) which bound to the separated single strands and reduced the rate of renaturation³⁶. In addition, exonuclease V unwinds linear duplex molecules in the presence of SSB protein³⁷.

The substrate used in the assay comprised ³²P-labelled complementary strand fragments annealed to ³H-labelled Φ X174 viral (+) strands (Fig. 2*a*). Figure 3 demonstrates that helicase I in the presence of ATP and SSB protein resulted in strand separation; *a* represents the sedimentation at neutral pH of the starting substrate. Although the ³²P-labelled DNA was only 194 nucleotides long, it sedimented rapidly because it was

annealed to the intact Φ X174 DNA. Figure 3*b* shows the results of incubation of 2 nmol DNA with 1.65 μ g helicase I for 30 min. The reaction mixture was then applied to the top of a neutral sucrose gradient and centrifuged. As can be seen, a portion of the annealed ³²P-DNA (46%) sedimented more slowly, indicating that strand separation had occurred. When SSB protein was omitted (Fig. 3*c*), only a small amount of strand separation (<10%) was observed. Omission of ATP or helicase I gave results identical to those in Fig. 3*c*, that is, no strand separation was observed. Very similar results were obtained when helicase I was replaced by helicase II (data not shown). These results are consistent with published data for helicase-catalysed strand separation^{31,32}.

In parallel experiments using identical conditions, the efficacy of recA protein in the strand separation assay was investigated. Figure 3*d* shows the results obtained after incubation of 2 nmol of substrate DNA (Fig. 2*a*) with 20 μ g recA protein for 30 min, in the presence of SSB protein, as described above. The ³²P-labelled DNA remained annealed to the intact Φ X174 DNA which indicated that recA protein did not separate the strands. Figure 3*e* shows the results of a similar reaction from which SSB protein had been omitted. Reactions were also done using recA protein in the range 2–200 μ g, with or without SSB protein or ATP, or where ATP was replaced with 0.5 mM γ -S-ATP, but no strand separation was detected in any of these conditions (data not shown). We also investigated the possibility that homologous ssDNA might stimulate strand separation by recA protein. ³H-labelled Φ X174 (+) ssDNA was sonicated briefly to produce DNA fragments of average length 400 nucleotides, as estimated by sedimentation. Figure 3*f* shows the results of incubation of 2 nmol of substrate DNA and 4 nmol homologous (+) fragments (Fig. 2*b*) with 40 μ g recA protein and ATP for 30 min as described. Similar results were obtained when the reaction mixture was supplemented with SSB protein (data not shown). No separation of the ³²P-labelled fragment from intact Φ X174 circles by recA protein was observed in conditions in which the separation of 10% of the fragments would have been easily detected.

To determine whether helicase-catalysed unwinding could be inhibited by the presence of recA protein, we examined its effect on the helicase II reaction in conditions of limiting helicase enzyme. Figure 4*a* represents the sedimentation of the starting substrate and *b* the results of incubating 2 nmol of substrate DNA (Fig. 2*a*) with 2 μ g SSB protein and 2.4 μ g helicase II for 30 min at 37 °C. The reaction mixture was then sedimented in neutral sucrose. At this concentration of helicase II, 32% of the annealed DNA was separated from the substrate DNA and sedimented more slowly. Addition of 20 μ g recA protein to an identical reaction mixture caused a slight increase in the extent of separation (from 32 to 48%, Fig. 4*c*), indicating that no inhibitory factors were present.

The results shown in Figs 3 and 4 show that recA protein did not act as a helicase. In similar conditions helicases I or II separated the annealed fragment from the single-stranded circle. The addition of recA protein to helicase II did not inhibit the reaction.

Formation of joint molecules by recA protein

The DNA substrate used in these experiments comprised a single-stranded circle with an annealed single-stranded fragment (Fig. 2*a*), and is similar to a gapped duplex. Because recA protein produced joint molecules from intact and gapped duplex molecules^{26,27}, we investigated whether the substrate with a very short duplex region (Fig. 2*c*) also formed joint molecules in the presence of recA protein. Nitrocellulose filters were used in the assay²⁶ because they allow form I DNA to pass but retain both substrate DNA and joint molecules due to their partially single-stranded nature.

To study the formation of joint molecules we incubated 0.2 nmol ³²P-labelled form I Φ X174 DNA and 1.7 nmol sub-

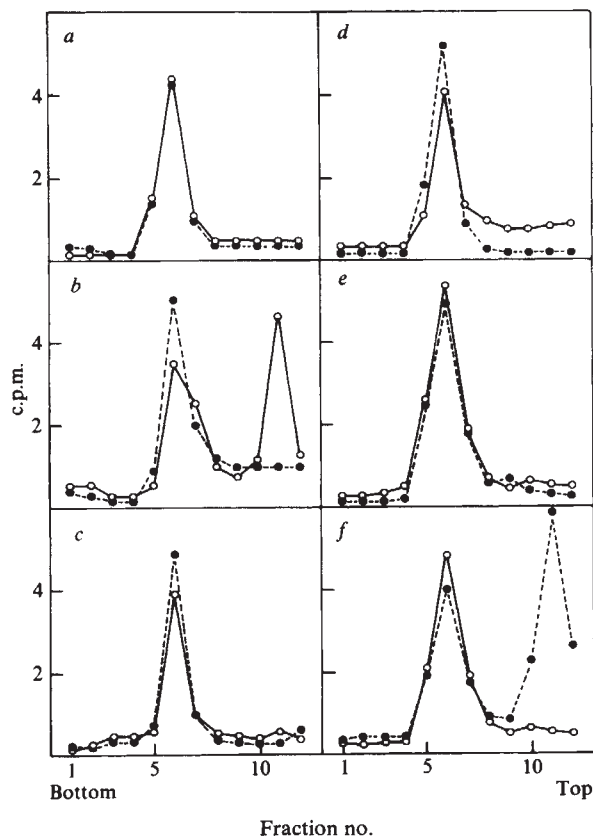


Fig. 3 Neutral sucrose sedimentation profiles showing the separation of a ^{32}P -labelled ΦX174 fragment from ^3H -labelled ΦX174 single-stranded circular DNA by helicase enzymes. The standard reaction mixture was used: *a*, without enzyme; *b*, with helicase I; *c*, with helicase I but without SSB protein; *d*, with *recA* protein; *e*, with *recA* protein but without SSB protein; *f*, with *recA* protein and excess homologous ^3H -labelled (+) single-stranded ΦX174 fragments. Sedimentation is to the left. The substrate for the unwinding assay was prepared by digesting ΦX174 form I ^{32}P -labelled DNA with *HaeIII* restriction enzyme (Bethesda Research Laboratories) and separating the products on a 5% polyacrylamide slab gel. The 194-base pair fragment was eluted from the gel, concentrated by ethanol precipitation and denatured by heating, followed by rapid cooling. The fragments were annealed with the (+) strands of ΦX174 ^3H -labelled phage DNA. Fragments not bound to the (+) strands were removed by sucrose gradient centrifugation. *RecA* and SSB proteins were purified as described elsewhere^{27,30}. The standard reaction mixture contained in a total volume of 100 μl , 2 nmol of substrate DNA, 20 mM Tris-HCl, pH 7.5, 25 mM MgCl_2 , 20 mM NaCl, 0.1 mM EDTA, 2 mM dithiothreitol, 2 mM ATP, 1.5 μg bovine serum albumin, 2 μg SSB protein and enzyme to be tested. Incubation was for 30 min at 37°C. The reaction was stopped by the addition of EDTA to 25 mM and SDS to 1% and, after dilution with 0.2 M NaCl to 200 μl , the mixture was sedimented through 5 ml of 5–20% neutral sucrose (10 mM Tris-HCl, pH 7.5, 1 M NaCl, 10 mM EDTA). Sedimentation was carried out in a Beckman SW 50.1 rotor at 45,000 r.p.m. and 20°C for 135 min. Fractions collected from the gradients were counted for radioactivity in Formula 963 scintillation fluid (NEN). $\circ$, ^{32}P , $\times 10^{-2}$; $\bullet$, ^3H , $\times 10^{-3}$.

strate DNA (Fig. 2c) in the presence of 40 μg of *recA* protein, treated the products with SDS and filtered them through nitrocellulose. The filters retained ~20% of the duplex DNA (Table 1, experiment 1). In control reactions done in the absence of any proteins or with SSB protein alone, a background of 10% of the input duplex DNA was retained. However, in the presence of both 40 μg of *recA* protein and 2 μg of SSB protein, almost 40% was retained. These results show that *recA* protein forms joint

molecules from the annealed substrate and form I DNA in conditions in which strand separation was not detected. As in other pairing reactions promoted by *recA* protein, ATP and Mg^{2+} were required.

The requirement for homology between molecules was also demonstrated with the filter binding assay. Joint molecules were not detected when the annealed substrate DNA and non-homologous ^3H -labelled form I plasmid DNA were incubated with *recA* and SSB proteins (Table 1, experiment 2). We also investigated whether single-stranded circular ΦX174 DNA, without the annealed fragment, could pair with intact circular duplex DNA (Fig. 2d). Experiments 3 and 4 in Table 1 show that incubation of single-stranded ΦX174 DNA with either ^{32}P -labelled form I ΦX174 or ^3H -labelled form I plasmid DNA (Fig. 2d) in the presence of *recA* and SSB proteins did not lead to retention of the duplex DNA. This shows that the partially single-stranded and partially duplex character of the substrate is needed for the formation of joint molecules.

What is the nature of the joint between the two molecules? A joint molecule could be formed by the transfer of one end of the annealed fragment to the duplex. The mechanism responsible for such a reaction might sometimes transfer the entire short fragment and so produce a D-loop which would be detected as a joint molecule in the filter binding assay. To investigate whether the observed binding of the form I DNA was due to the transfer of the ^3H -labelled fragment to the ^{32}P -labelled duplex (to form a D-loop), the reaction was stopped at the end of incubation by the addition of 1 mM $\gamma\text{-S-ATP}$ and the form I DNA was cleaved into small fragments with *HaeIII* restriction enzyme. After protein removal by treatment with proteinase K and SDS, the products were sedimented through neutral sucrose. The ^3H -labelled fragments sedimented with the single-stranded circular DNA and none (<3%) was detected at the position of the ^{32}P -labelled restriction fragments which sedimented more

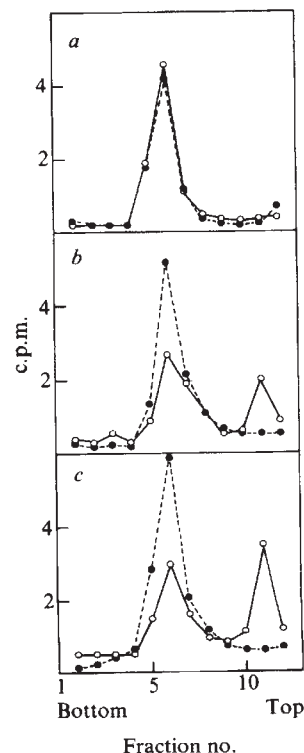


Fig. 4 *RecA* protein does not inhibit helicase II-catalysed strand separation. The strand separation assay was the same as that described in Fig. 3 legend. The standard reaction mixture was used: *a*, without enzyme; *b*, with helicase II; *c*, with helicase II and *recA* protein. $\circ$, ^{32}P , $\times 10^{-2}$; $\bullet$, ^3H , $\times 10^{-3}$.

slowly. This experiment indicates that no detectable transfer of the annealed fragments to the form I DNA occurred, and will be described in greater detail elsewhere³⁸.

These results indicate that recA and SSB proteins promote the formation of joint molecules between intact duplex DNA and homologous single-stranded molecules that contain a short duplex region (Fig. 2c). This pairing reaction occurred in conditions similar to those used in the assay for helicase activity.

Homologous pairing between gapped and intact duplexes

Because most models for genetic recombination^{1-5,8} assume that the first steps in homologous pairing involve strand unwinding and the formation of a heteroduplex between the unwound strand and the complementary strand of the homologous duplex (Fig. 1a), we looked for evidence that recA protein could promote strand separation.

Using single-stranded circular DNA with an annealed fragment (Fig. 2a), we were unable to detect any helicase activity of recA protein, with or without the presence of SSB protein (Fig. 3). Furthermore, homologous pairing does not seem to require extensive strand separation because treatment of gapped molecules with psoralen and light to produce a minimum of 1 cross-link per 60 base pairs did not inhibit the formation of joint molecules²⁷. However, we found that recA protein, which is known to promote homologous pairing between gapped circular and form I DNA^{26,27}, was also able to induce homologous pairing between form I DNA and ssDNA carrying an annealed fragment (Fig. 2c, Table 1), the yield being increased by SSB protein. Although the fragment was only 392 nucleotides long, it was essential for pairing.

In this reaction, the joint molecules could be formed through the transfer of one end of the fragment to the form I duplex while the other end remained bound to the single-stranded circle, thus linking the molecules as in Fig. 1A. However, it is difficult to see why the transfer of a short fragment should stop before completion. It is more likely that the transfer would go to completion and the fragment be completely assimilated into the duplex, but no such transfer was detected. If the reaction was not driven to completion, the intermediate structure would presumably dissociate through branch migration³⁹.

Although we did not detect strand transfer from the annealed substrate when form I DNA was added, we have found in other reactions that recA protein promotes a strand exchange reaction if the form I DNA is replaced by blunt-ended duplex fragments

that are homologous to and overlap the ends of the annealed fragment³⁸. This exchange might take place by a reaction similar to that described for the formation of D-loops from single-stranded circular and linear duplex DNA^{38,40}.

How are these observations to be reconciled? We suggest that recA protein can process some DNA substrates via D-loop formation involving strand transfer, while it may process others by four-stranded complementary pairing (Fig. 1B), perhaps similar to that proposed from studies with DNA models^{41,42}, or by another mechanism involving three-stranded side-by-side pairing. Complementary hydrogen bonding may occur between two duplexes without strand separation and involves a four-stranded helical structure with contacts in the major groove⁴². We attribute the formation of joint molecules between duplex and gapped circles, described here and in recent papers^{26,27}, to these latter mechanisms.

How does recA protein promote homologous pairing between two duplex molecules? The only structure known to cause exchanges with a high efficiency in intact cells is a duplex containing a post-replication gap²⁹. Purified recA protein binds cooperatively to single-stranded but not duplex DNA. It also binds to gapped duplex DNA and shows high ATPase activity, almost as great as when bound to ssDNA³⁰. To explain this we suggest that it binds initially at the gap and spreads along the adjacent duplex (Fig. 1B, I), forming a bound state which may be transient as ATP is being hydrolysed. Homologous pairing can take place in conditions in which the DNA is loaded with recA protein. The initial nonspecific contact between gapped and intact molecules (Fig. 1B, II) presumably leads to a search for homology and the formation of joint molecules with the base sequences aligned (see XYZ in Fig. 1B, III). Once homologous contacts are made, the DNA molecules are held together by hydrogen bonds which seem to be stable, even when deproteinized with SDS and proteinase K but are, however, alkali labile^{26,27}.

The joint molecules formed by homologous pairing may then be acted on by the cutting in *trans* enzymes^{43,44}, opening the way for strand exchanges^{38,40} and formation of recombinants. Future work will determine the validity of this proposal for homologous pairing, the details of the structures involved in the joint molecules, and the way in which they are cut and joined to produce recombinants.

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LETTERS

H₂ emission in the EUV spectrum of T Tauri and Burnham's nebula

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Emission in the Lyman bands of H₂ has been detected in an extreme ultraviolet (EUV) spectrum of T Tauri and its adjacent nebula, obtained with the international UV explorer (IUE) satellite. We report here that the emission is in a progression which can be excited through fluorescence with the red wing of the H Ly α line, as found also in the solar atmosphere¹. The observed EUV fluxes, absence of other progressions and the flux in the previously observed² 1–0 S(1) IR line support a model where collisional rather than radiative excitation leads to an excitation temperature of $\geq 2,000$ K.

T Tau is a pre-main-sequence star, with a photospheric absorption spectrum consistent with a spectral type of K0 or K1 according to Kuhl³. It is embedded in nebulosity, from which characteristic low-excitation lines are observed in the visible region of the spectrum⁴. Emission from the $v'' = 1-0$ S(1) line of molecular hydrogen at 2.12 μ m has also been detected², originating from a region ~ 5 arc s in extent centred on the star. The distance³ to T Tau is ~ 160 pc.

We observed T Tau on 4 November 1980 with the IUE satellite, from the ESA IUE ground station at Villafranca, Madrid. A short wavelength region (1,200–2,000 Å) spectrum was obtained at low spectral resolution ($\Delta\lambda \sim 6$ Å) with an exposure time of 372 min, using the large (21 $\times$ 9.5 arc s) aperture. The spectrum shows emission from both the star and nearby nebulosity which extends to the south. A long wavelength region (2,000–3,200 Å) spectrum was obtained at high spectral resolution ($\Delta\lambda \sim 0.2$ Å) with an exposure time of 176 min, also using the large aperture. Only the stellar emission lines of Mg II (multiplet 1) and Fe II (multiplet 1) are well exposed; many weak emission features are just detectable.

Figure 1 shows tracings through the short-wavelength spectrum of the star and nebulosity. Figure 2 shows the profiles of the stellar Mg II lines. The short wavelength spectra have been extracted from the GPHOT image by procedures discussed elsewhere⁵, and making use of the Rutherford-Appleton Laboratory IBM 360/195 computer. The long-wavelength spectrum is as provided by the IUE VILSPA processing system.

The strongest features in the short-wavelength spectrum may be attributed to transitions in O I, C II, C IV, Si II, Si III and C III

commonly observed in the EUV spectra of late-type stars. Some of these have been observed previously in a shorter exposure of T Tau⁶, and also in the T Tauri-type stars R U Lupi⁷ and R W Aur⁸.

In addition to these lines, emission features of lower intensity are observed at the wavelengths listed in Table 1. These are the only lines which can be positively detected in the spectrum of the nebulosity. We identify the lines listed in Table 1 as transitions in the Lyman bands of H₂. These particular lines have been observed previously in the EUV spectrum of a sunspot¹ and it would be difficult to make a positive identification of H₂ emission without this prior knowledge. The observed wavelengths correspond to those of the strongest H₂ lines seen in the solar spectrum, namely the P(5) and R(3) transitions in the $v' = 1$ to $v'' = 3, 6, 7$ and 8 bands. The laboratory wavelengths in Table 1 are taken from ref. 9. The 1–4 and 1–5 bands should be weaker, according to the calculated transition probabilities¹⁰. In the Sun

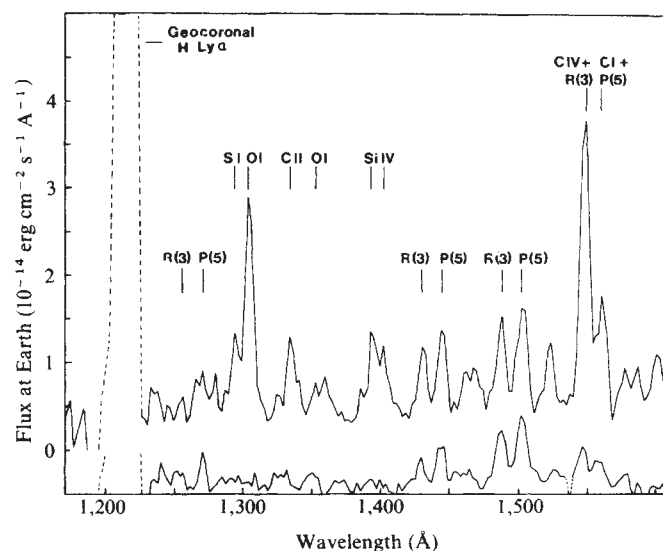


Fig. 1 Low resolution spectrum of T Tau in the region 1,200–1,600 Å. In addition to the common species indicated, the P(5) and R(3) lines of H₂ in the $v' = 1$ to $v'' = 3, 6, 7$ and 8 are observed. The upper spectrum originates in the star plus nebula in the line of sight. The lower spectrum originates in the adjacent nebulosity, and is plotted on a scale lower by 0.5×10^{-14} erg cm⁻² s⁻¹ Å⁻¹.

the lines are due to fluorescent emission from the upper level $v' = 1, J' = 4$, which is excited through the $v'' = 2, J'' = 5$ to $v' = 1, J' = 4$ transition, which lies ~ 26 cm⁻¹ to the red of the rest wavelength (1,215.67 Å) of the atomic H Ly α line. We propose that the same excitation process is occurring in the region around T Tau. The listed fluxes include the line of sight plus the adjacent nebulosity, the latter contributing about 30% of the total. (It is not possible to distinguish between stellar and nebular emission in the line of sight.)

The presence of this emission in the absence of a strong UV continuum has important implications for the excitation of H₂ molecule in such regions. For the observed fluorescent route to be effective, a substantial population of H₂ must reside in the excited $v'' = 2, J'' = 5$ level. It has been proposed that shock heating in addition to excitation by UV radiation is required to account for the intensities of lines of H₂, both around T Tau^{11,12} and in the Orion nebula^{13–15}. Detection of the S(1) 2.12- μ m line alone³ did not allow shock excitation to be distinguished from excitation by a strong EUV continuum^{16–18}. The new EUV observations favour a collisional excitation model, probably associated with shock heating. The likely relevance of H Ly α

Table 1 Lines of H₂ observed in T Tau

λ (Å)	Flux at Earth (erg cm ⁻² s ⁻¹)	v'	v''	Proposed identification	λ lab ⁹ (Å)
1,258	$\leq 4.2 \times 10^{-14}$	1	3	R(3)	1,257.80
1,272	8.6×10^{-14}			P(5)	1,271.93
1,431	1.0×10^{-13}	1	6	R(3)	1,431.01
1,446	1.4×10^{-13}			P(5)	1,446.13
1,490	1.8×10^{-13}	1	7	R(3)	1,489.57
1,505	2.3×10^{-13}			P(5)	1,504.79
1,547	3.6×10^{-13}	1	8	R(3) + C IV	1,547.35
1,562	1.7×10^{-13}			P(5) + C I	1,562.41

fluorescence in regions where excited H_2 exists has already been pointed out^{19,20}.

The $H\text{Ly}\alpha$ emission visible in the IUE spectrum is due mainly to geocoronal emission, and high resolution spectra will be required to investigate the stellar component. However, the $Mg\text{II}$ lines shown in Fig. 2 give some idea of the shape of the line profile. These lines show strong emission, with the centre of this emission shifted by 50 km s^{-1} to the red of the strong interstellar absorption feature which in this direction should itself be redshifted by $\sim 10\text{--}20\text{ km s}^{-1}$. As the star has a radial velocity²¹ of only 22 km s^{-1} , and the width of the $Mg\text{II}$ emission at the star will be at least the value of 120 km s^{-1} observed at the Earth, it can be argued that substantial flux should exist in the red wing of the $H\text{Ly}\alpha$ line, in particular at $1,216.05\text{ \AA}$, the wavelength at which fluorescent excitation takes place. An estimate of the $H\text{Ly}\alpha$ flux in the relevant wavelength range can be made by scaling from the $Mg\text{II}$ flux, which is typically a factor of ~ 4.5 larger than $H\text{Ly}\alpha$ in a variety of late-type stars²⁵. This gives a flux of $\sim 10^6\text{ erg cm}^{-2}\text{ s}^{-1}$ in the equivalent part of the $H\text{Ly}\alpha$ line.

Provided all transitions from $v' = 1, J' = 4$ are optically thin, the measured flux in the $P(5)$ line at $1,505\text{ \AA}$ can be used in conjunction with the expression for the emissivity given by Jordan *et al.*¹⁹ to give

$$\int N(2, 5) I_\nu dV \sim 3.0 \times 10^{33} \quad (1)$$

where $N(2, 5)$ is the population density (cm^{-3}) in the level from which excitation takes place, I_ν is the intensity of the $H\text{Ly}\alpha$ radiation field ($\text{erg cm}^{-2}\text{ s}^{-1}\text{ Hz}^{-1}$) at the nebula. Allowing for a geometric dilution factor in a medium optically thin to $H\text{Ly}\alpha$, I_ν can be related to I_ν^* , the intensity at the star. Then

$$I_\nu \sim 1.0 \times 10^{17}/R^2 \quad (2)$$

where R (cm) is the distance at which absorption by H_2 takes place.

The observed relative intensities of the transitions to $v'' = 3$ and 6 or 7 suggest that the lines to $v'' = 3$ are marginally optically thick. However, even if the transitions to $v'' \leq 3$ are optically thick, a probability of escape argument for lines from a common upper level shows that the enhancement of the optically thin transitions is only a factor of 1.7. In view of the larger uncertainties this correction has not been applied.

The IR observations show that the H_2 emission originates from a region around the star of $\sim 5\text{ arc s}$ ($1.2 \times 10^{16}\text{ cm}$) in total extent. The spatial distribution in the optical region and in our EUV spectrum is asymmetric and less than 10 arc s in extent. We adopt an emitting volume $F4\pi R^2 dl$, where F is some fraction of the spherical shell of radius R . The column density in the 2, 5 level is then

$$\int N(2, 5) dl \sim \frac{1}{F} 2.4 \times 10^{15} \text{ cm}^{-2} \quad (3)$$

Similarly the observed flux in the $S(1)$ transition and transition probability²² can be used to give

$$R^2 \int N(1, 3) dl \sim \frac{1}{F} 2.2 \times 10^{47} \quad (4)$$

Equations (3) and (4) then give $N(2, 5)/N(1, 3)$ as a function of R , assuming that the emitting volumes are the same. The value

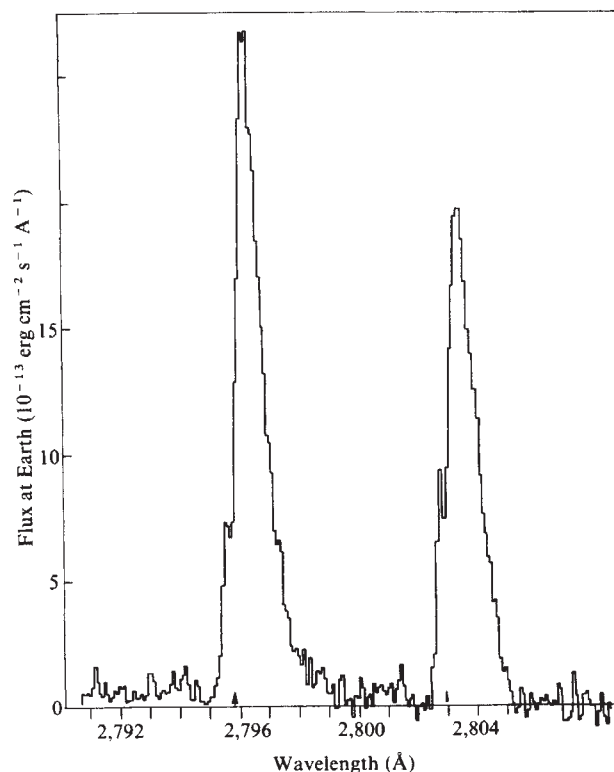


Fig. 2 High resolution spectrum of the $Mg\text{II}$ resonance lines in T Tau. The arrows mark the rest wavelength at the star. Narrow interstellar lines appear in broad asymmetric stellar lines.

of the excitation temperature, T_{exc} , required to give this population ratio may then be found.

Table 2 gives values of R , the radius of the shell, for temperatures between 1,000 and 3,000 K. These are somewhat smaller than the value of $6 \times 10^{15}\text{ cm}$ found from the IR observations. A larger flux in the stellar $H\text{Ly}\alpha$ emission would result in a larger value of R . Even allowing for an order of magnitude underestimation of I_ν^* would lead to $T_{\text{exc}} \geq 1,500\text{ K}$.

A further estimate of T_{exc} can be made by using the observed upper limit to the flux at $1,365.7\text{ \AA}$, where the strongest member of the $P(1)$ progression, which can be excited by $H\text{Ly}\beta$, should occur²³. The ratio of the flux at $1,365.7\text{ \AA}$ to that in the $1,505\text{ \AA}$ line gives

$$N(2, 5)/N(0, 1) \geq 2 \times I(\text{Ly}\beta)/I(\text{Ly}\alpha).$$

Using $I(\text{Ly}\beta)/I(\text{Ly}\alpha) \sim 10^{-2}$, as in the Sun, results in $T_{\text{exc}} \geq 2,700\text{ K}$.

The mass column density (from equation (3) with $F \leq 1$), emitting mass and lower limits to $N(H_2)$ for a filled shell, are also given in Table 2. These mass column densities are consistent with lines to $v'' \leq 3$ being optically thick. Solutions with $T_{\text{exc}} \leq 1,000\text{ K}$ are excluded by the upper limit imposed by known⁴ visual extinction to the star and nebula.

Finally we may compare the results of Beckwith *et al.*² who adopted Kwan's model¹⁵ for excitation by a low velocity shock and subsequent emission through the $1\text{--}0\text{ S}(1)$ line. With $v_{\text{shock}} \sim 15\text{ km s}^{-1}$ they find $N(H_2) \sim 2 \times 10^4\text{ cm}^{-3}$. Adopting recent calculations of dissociation speeds²⁴, the upper limits to the shock velocity can be found as a function of $N(H_2)$ and are given in Table 2. In the absence of a strong EUV continuum it would be difficult to destroy the H_2 once formed.

We conclude that the EUV observations are consistent with a model where H_2 is at an excitation temperature of $\geq 2,000\text{ K}$, and that the basic excitation mechanism is collisional rather than radiative.

Table 2 Parameters of the emitting region as a function of T_{exc}

T_{exc} (K)	R (10^{15} cm)	$\int N(H_2) dl$ (cm^{-2})	$N(H_2)$ (cm^{-3})	$\mathcal{M}$ ($\mathcal{M}_\odot$)	$v_{\text{shock},1}$ (km s^{-1})
1,000	0.38	$\geq 2.0 \times 10^{21}$	$\geq 5.3 \times 10^6$	5.6×10^{-6}	<21
1,500	0.91	$\geq 5.7 \times 10^{19}$	$\geq 6.3 \times 10^4$	9.9×10^{-7}	<22
2,000	2.10	$\geq 4.9 \times 10^{18}$	$\geq 2.3 \times 10^3$	4.4×10^{-7}	<32
3,000	3.60	$\geq 7.9 \times 10^{17}$	$\geq 2.2 \times 10^2$	2.0×10^{-7}	<51

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A water vapour maser in the Large Magellanic Cloud

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Surveys of the Large Magellanic Cloud (LMC), the closest satellite galaxy to our Solar System, for water vapour sources of measurable intensity have so far yielded negative results. These searches, by Johnston *et al.*¹ and Kaufmann *et al.*², with detection limits of about 150 and 20 Jy ($1 \text{ Jy} = 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$), respectively, suggest that any water vapour masers that exist in the LMC are much weaker than the sources recognized in our own Galaxy and in several more distant galaxies^{3–5}. We now report the results of a survey using the 45-ft Itapetinga radio telescope which shows the presence of a water vapour maser in the N159 region of the LMC.

The survey was carried out in 1979–80. Upper detection limits of about 1.2 Jy were attained with the use of long integration times and a maser amplifier front-end, the total system temperature ranging from 250 to 400 K. Spectral information was provided by a 47-channel spectrometer, 100-kHz bandwidth filter bank, giving a velocity resolution of 1.35 km s^{-1} . Two vertically polarized feed horns were used in beam-switching the on-off observing mode. Each independent observation was taken with 30 min integration. We selected observations made in good weather conditions and our data were corrected for atmospheric attenuation effects by assuming an average atmospheric optical depth of 0.2. The data were also corrected for random attenuation and elevation gain dependence.

We concentrated our search for the $J = 6_{16} - 5_{23}$ transition of the water maser line ($f = 22235.08 \text{ MHz}$) in two of the strongest H(II) regions in the LMC⁶—N157 (ref. 7, 30 Doradus) and N159—and in two dark nebulae⁸—Hodge 47 and Hodge 52. Huggins *et al.*⁹ studied these four regions in their carbon monoxide survey.

Water vapour emission was detected in only one of these four positions, N159, and its spectrum is shown in Fig. 1. It presents two components, one 8 km s^{-1} wide with a narrow feature of 7.2 Jy at 233.6 km s^{-1} , and the other with a feature of 3.2 Jy at 213.9 km s^{-1} .

It is interesting that the water maser we discovered in N159 coincides with the lowest colour temperature far-IR source detected by Werner *et al.*¹⁰ in the LMC—30 Doradus—which is also the strongest source. A large CO cloud, 24 pc in diameter, was found in this direction⁹. Other molecules such as H_2CO (ref. 11), OH (ref. 12) and CO (ref. 9), have been discovered at this position in the LMC, with velocities almost coincidental with the H_2O velocity.

Regarding the intensity of this maser, it would be a strong source of $8 \times 10^4 \text{ Jy}$ if placed at the distance of the Orion Nebula, but when compared with the W49 masers, it is much weaker in terms of total luminosity¹³.

A negative result was obtained for the 30 Doradus nebula, probably due to the fact that its core contains a large number of young OB stars¹⁴ responsible for the ionization of the molecules.

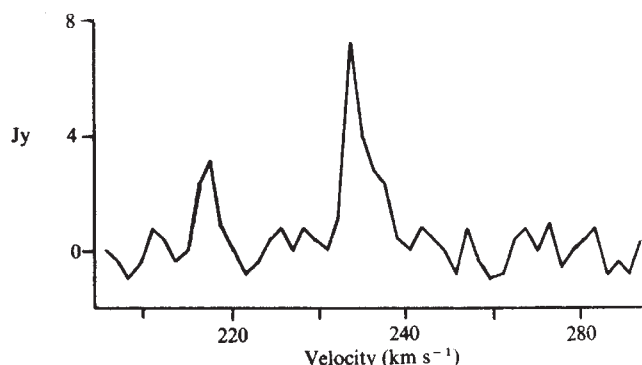


Fig. 1 Water vapour spectrum of N159 (RA 05 h 40 min 24 s DEC. $-69^{\circ}46'0''$, 1950.0). The main feature appears at 233.6 km s^{-1} with respect to the Local Standard of Rest.

Hence, one should search for water farther away from its nucleus. Hodge 47 and 52 also showed no line emission.

Our search for water masers in the direction of the Large and the Small Clouds will continue, and we hope that the results improve our knowledge not only of masers but also of our closest irregular Magellanic-type galaxies.

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Discrete chorus emissions observed at a low latitude ground station

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Discrete chorus emissions observed for the first time at the low latitude ground station Bichpuri, Agra (geomagnetic lat. $17^{\circ}12'N$, $L \sim 1.1$) are reported here. The emissions are of the riser type and occurred in the narrow frequency range 1.7–3 kHz. We show that these emissions are generated in the equatorial plane near $L = 1.2$ as a result of cyclotron resonance between whistler mode waves and the inner radiation belt relativistic electrons of energy 3–5 MeV, and propagated to the low latitude ground station under the influence of the horizontal density gradients of the equatorial anomaly.

Using conventional equipment consisting of an antenna, transistorized pre and main amplifiers and a magnetic tape recorder, we started routine observations of whistlers at a low latitude ground station of Bichpuri between January 1979 and July 1979. Bichpuri is in a noise-free rural area ~ 11 km west of Agra. The observations were made at night (18.00–06.00 h LT) during the first 10 min of every hour, but, the duration of the recording was increased whenever the whistler activity was found to be high. On 12 January 1979, which was a magnetically quiet day ($K_{pmax} = 3$), we observed nine discrete rising emissions between 19.25 and 20.40 h LT. Some of these emissions, shown in Fig. 1, occurred in a narrow frequency range between 1.7 and 3 kHz with the rate of change of frequency with time (df/dt) between 2 and 3 kHz s^{-1} . The 10 ms structure in the spectra of these emissions is due to the instrument. Such structures (time interval ~ 1.5 s) have also been observed in high latitude periodic emissions reported by Helliwell¹ (Fig. 7.21). Figure 1a, where two rising emissions are shown, consisted of four emissions; the other two occurred somewhat later. However, all four emissions are shown on a different frequency–time record in Fig. 2. An interesting feature of these emissions is that the time interval between the consecutive emissions is approximately in a geometrical progression. Further, the frequency ranges of first

and third emissions are slightly different from those of the other two emissions.

Hitherto, discrete chorus emissions have been observed only at middle and high latitude ground stations. The lowest latitude ground station where such emissions were received is Chilbolton², Hampshire, UK (geomagnetic lat. $49.8^{\circ}N$, $L = 2.4$). The emissions observed by us could not have been caused by a fault in the equipment because several whistlers, with dispersions in the range $19\text{--}35$ s^{1/2} were also observed by the same equipment. As these riser emissions differ markedly in frequency and df/dt from those of the riser whistlers observed earlier at the low latitude ground station of Gulmarg³ (geomagnetic lat. $24^{\circ}10'N$), the possibility that these emissions are also riser whistlers is ruled out. Further, the possibility that these emissions are high latitude discrete chorus emissions which have propagated to our low latitude ground station in the Earth-ionosphere wave-guide mode of propagation³ is also ruled out because there is a strong absorption band in the frequency range 2–5 kHz. The possibility that these emissions are generated at high L -values in the vicinity of the plasmapause and propagated to our ground station after successive magnetospheric reflections in a manner similar to those of the ELF hiss observed by satellites in the inner zone^{4,5} does not seem to be tenable. This is because, at the frequency of these emissions, the attenuation losses during various magnetospheric reflections would be high and the waves could not be detected on the ground. Although the exact losses have not been calculated, a rough estimate can be made from the work of Kimura⁶. He has shown from ray tracing computations in a model ionosphere that the amount of attenuation suffered by the waves of frequency 1 kHz from 300 km at $30^{\circ}N$ reaching $L = 3$ after 12 successive reflections is ~ 6 dB. If we include ~ 4 dB of losses suffered in the lower region of the ionosphere, then the total loss will be ~ 10 dB. To reach the plasmapause near $L = 4$, the waves have to undergo some more reflections and consequently suffer more attenuation than 10 dB. As the attenuation increases with frequency, the waves of the chorus emissions generated near the plasmapause would suffer much higher attenuation than 10 dB and may not be observed on the ground. Further, at the base of the F-region ionosphere, the wave normal angles of these waves are such that the downward waves are unlikely to penetrate the lower ionosphere and reach the ground.

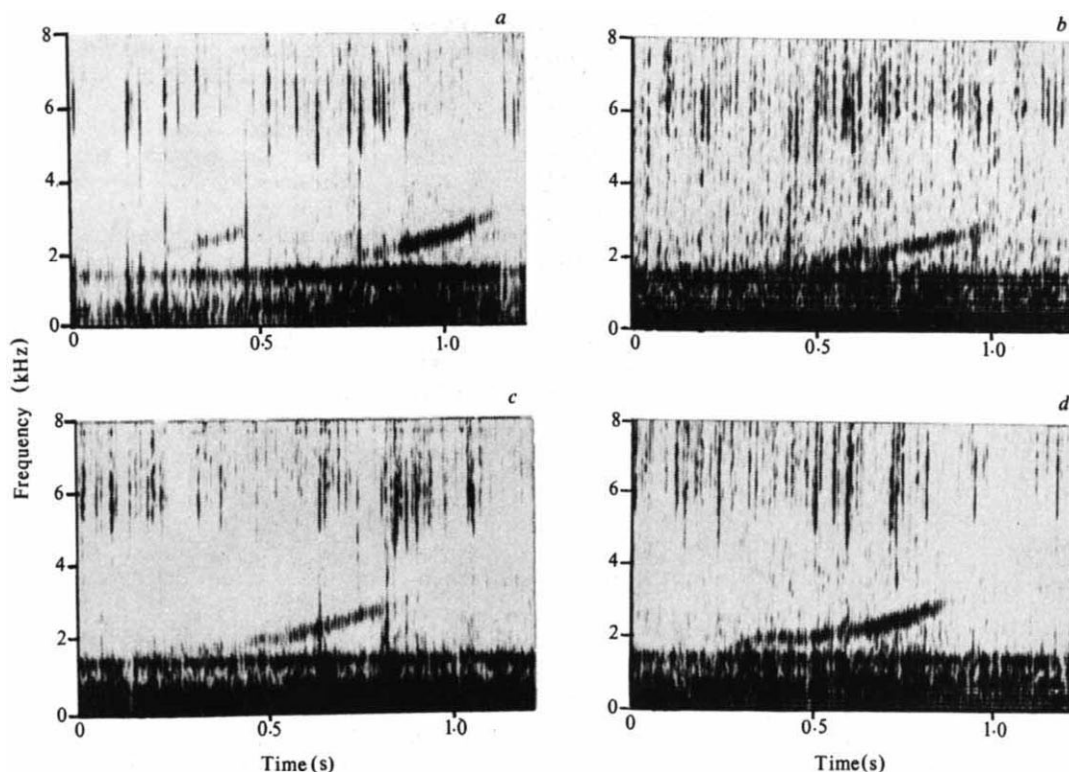


Fig. 1 Discrete chorus emissions observed at the low latitude ground station of Bichpuri. a, 19.27 IST; b, 19.54 IST; c, 19.48 IST; d, 19.53 IST.

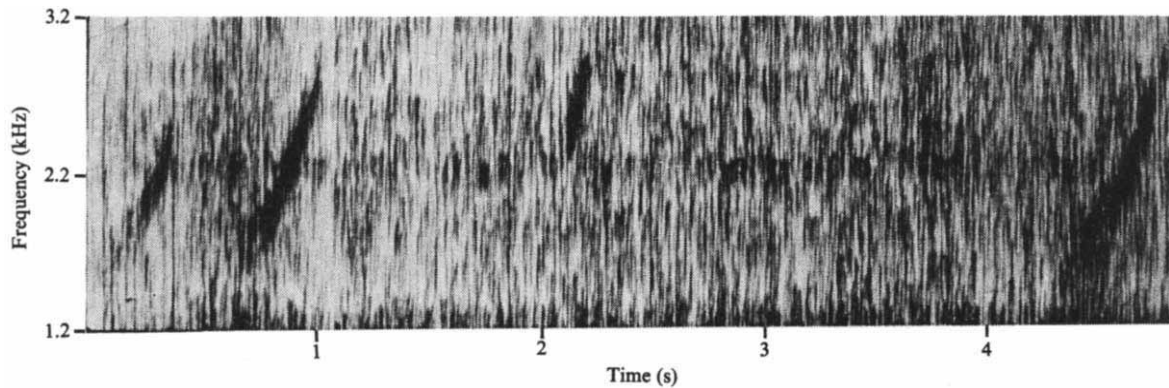


Fig. 2 Risers with time interval increasing in geometrical progression.

Thus we believe that these emissions are generated in the equatorial plane in the inner zone radiation belt ($L \sim 1.2$). Cyclotron resonance between whistler mode waves and the inner zone radiation belt electrons seems to be the possible generation mechanism for two reasons: (1) this mechanism has been found to account for the frequency-time spectra of middle and high latitude discrete chorus emissions adequately²; and (2) it also occurs in the inner radiation belt⁷. Below $L = 1.2$ electron losses are dominated by atmospheric scattering^{7,8} resulting in the small life time and density of the energetic electrons and hence the emissions observed by us cannot be generated at the top of the field line corresponding to Bichpuri. These emissions cannot be generated at L -values > 1.2 for the reasons to be stated later. To test the cyclotron resonance as the possible generation mechanism for these emissions, we calculated the resonant energy of the involved electrons and growth rates at $L = 1.2$ in the equatorial plane. The resonant energies for various frequencies of the emissions were calculated from the expression⁵.

$$E_{11} = (\gamma_r - 1)m_0 c^2 \quad (1)$$

in which m_0 is the rest mass of electron, c , the velocity of light in vacuum and γ_r , the relativistic factor to be obtained from the relation⁵

$$\gamma_r^2 - 1 \equiv \left(\frac{\bar{\Omega}}{\omega_p}\right)^2 \left(\frac{\bar{\Omega}}{\omega}\right) \left(1 + \frac{\Omega^+}{\omega}\right) \quad (2)$$

where $\bar{\Omega}$ is the electron gyrofrequency, Ω^+ , the proton gyrofrequency, ω_p , the plasma frequency and ω , the wave frequency. The plasma frequency ω_p was calculated from the ionospheric model of Singh⁹ which yielded an electron density of 8.13×10^3 electrons cm^{-3} at $L = 1.2$. The calculated resonant energies, of the order of 3–5 MeV, are shown in Fig. 3a. For calculating the growth rate we followed expression (2.20) of Kennel and Pet-

schek¹⁰, which, for the case $\omega \ll \bar{\Omega}$, reduces to

$$\gamma = \pi \bar{\Omega} \eta A \quad (3)$$

where η is the ratio of the density of energetic electrons to that of thermal electrons and A , the pitch angle anisotropy. To obtain the density of energetic electrons at $L = 1.2$ we considered observed intensity versus L -value curves (energy > 1 MeV) given by Katz¹¹ for the inner zone radiation belt. The curve indicated a flux of 3×10^6 electrons $\text{cm}^{-2} \text{sr}^{-1} \text{s}^{-1}$ at $L = 1.2$ which yielded a density of 3–5 MeV electrons $\sim 1.26 \times 10^{-3}$ electrons cm^{-3} . A was calculated from the relation¹⁰ $A = \frac{1}{2} / \log_e(\frac{1}{\alpha_0})$ (α_0 being the equatorial loss cone angle, mirror height = 100 km) and found to be 2.08 which is approximately the same as calculated by Ashour-Abdalla¹² at $L = 1.2$ ($P = 250$). If we assume a pitch angle distribution of the form $\sin^m \phi$ ($m = 4$, and ϕ is the pitch angle) and compare the calculated pitch angle versus intensity curve with that of Katz¹¹, the two distributions are found to be nearly the same. Thus the value of the anisotropy $A = 2.08$ at $L = 1.2$ is justified. By substituting the values of $\bar{\Omega}$, η and A in equation (3), the growth rates for various frequencies of the emissions were calculated and found to be $\sim 3 \text{ rad s}^{-1}$. This result indicates significant wave amplification.

Now we consider how the chorus emissions were propagated to our low latitude ground station. Suitable ducts do not exist in the low latitude ionosphere and non-ducted waves are not observed on the ground. However, Bullough *et al.*¹³ have reported the observation of VLF and ELF emissions in the Ariel 3/4 satellites inside the equatorial anomaly region. The equatorial anomaly is a well-established afternoon-evening phenomenon. Thus it is possible that the low latitude chorus emissions were propagated to our ground station under the influence of the diminishing phase of the equatorial anomaly. To verify this we calculated ray paths for the frequencies of the chorus emissions from $L = 1.2$ to the base of the F-region ionosphere (120 km) in an ionospheric model representing the diminishing phase of the equatorial anomaly. The diminishing phase of the anomaly was assumed to have a constant electron density distribution within $\pm 5^\circ$ of the equator instead of a trough at the equator. This distribution was obtained from the model $N = N_\infty \exp \alpha (\sin \theta - \beta)$ of Singh⁹ where the value of α was taken to be 11 for the negative horizontal density gradient between 22° and 5° and zero for no ionization variation between 5° and the equator. The ray paths computed for the frequencies of the observed chorus with the initial wave normals along the geomagnetic field line at the equator ($\Delta_0 = -90^\circ$) arrived within 18.5° and 19° latitudes at 120 km, with the final wave normals almost vertically downward ($\Delta_r \approx 178^\circ$) indicating that the corresponding waves would penetrate the lower ionosphere and be observed on the ground. One of the ray paths for the frequency of 3 kHz is shown in Fig. 3b. The ray paths computed from higher L -values of 1.24 and 1.47 arrived at 24° and 45° latitudes respectively with the wave normals not vertically downward. Such waves would not be observed on the ground. Thus the observed chorus emissions were generated at $L = 1.2$ and

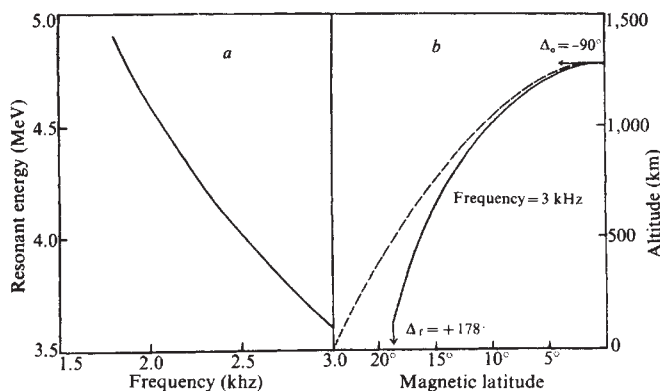


Fig. 3 a, The computed resonant energies at $L = 1.2$. b, Ray path for the frequency of 3 kHz. Δ_0 and Δ_r are the initial and final wave normal angles (angles between the wave normal and the vertical, measured from the vertical, positive in a clockwise direction).

propagated to the ground station under the influence of the favourable density gradients of the equatorial anomaly. The growth of these emissions at 1.7 kHz is due to the resonance between the energetic electrons and whistler mode waves of frequency lower than 1.7 kHz and termination of emissions at ~3 kHz is due to the absorption by the thermal background plasma in the interaction region¹⁴. The detailed mechanism in which the four emissions of Fig. 2 were generated with two of them having different upper and lower cutoff frequencies is under investigation and the result will be published elsewhere.

The occurrence of natural whistlers and emissions is a rare phenomenon at our ground station. During the 7 months of observation, we recorded whistlers on four occasions and emissions on one occasion only. The rare occurrence is believed to be due to non-availability of suitable ducts in the low latitude ionosphere. The emissions observed on 12 January 1979 between 19.25 and 20.40 h LT are due to fortuitous presence of favourable density gradients of the equatorial anomaly in the low latitude ionosphere. These emissions, although received only once at our ground station, indicate their origin in cyclotron resonance at low *L*-values in the ionosphere. Unfortunately, satellite data on inner zone electrons corresponding to the period during which the emissions were observed are not available to confirm the result. However, more data on these emissions and simultaneous satellite data on energetic electrons would be useful for studying the pitch angle scattering and precipitation of energetic particles as a result of cyclotron resonance at low *L*-shells. We have therefore continued the observations of these emissions in our ground station.

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High propagation rates of explosions in large volumes of gaseous mixtures

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To account for the level of damage observed following unconfined vapour cloud explosions of the kind that occurred at Flixborough in 1974, flame speeds of the order 10^2 m s^{-1} have had to be postulated^{1,2}. Detonations (in which reaction is initiated by adiabatic compression in a shock wave) are not thought to be involved, whilst deflagrations (in which molecular and turbulent transport processes from the flame bring the reactants to a state of rapid reaction) do not seem capable of producing sufficiently high velocities. We suggest here that radiation from a large enough body of burnt products could be sufficient to produce multi-point initiation ahead of the flame by igniting particles, especially those consisting of loose aggregates of fine fibres. Although we thought initially that the hazard would chiefly be due to particles of flammable materials, such as cotton waste and other 'fluffy' aggregates of cellulosic substances, we show below that non-flammable materials are also hazardous.

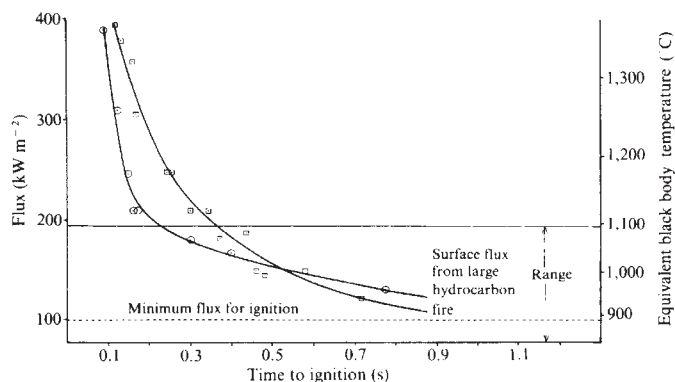


Fig. 1 Minimum ignition times for propane-air (~3.8% propane) ignition by Kaowool (○) and cotton wool particles (□).

Whilst the emissivities of combustion products are very small at optical path lengths encountered in normal practice, they become appreciable for the very large clouds of burnt and burning gases characteristic of vapour cloud explosions. The black-body radiation corresponding to stoichiometric flame temperatures is of the order of 1 MW m^{-2} . In view of the difficulties of arranging and monitoring large scale explosions of premixed reactants, existing measurements have generally been confined to large experimental fires³⁻⁴, usually burning pools of liquid fuels (see, however, ref. 5). These are diffusion flames in which burning does not occur uniformly throughout the gas but is confined to a relatively thin peripheral layer. Radiation fluxes measured near such flames are of the order of $100\text{--}200 \text{ kW m}^{-2}$.

It is easy to show that quite sporadic ignitions ahead of the main front would yield a sufficiently enhanced flame speed. Defining flame speed in terms of the rate of consumption of a given volume of reactants—which also defines the increased rate of generating volume of hot products—multiple ignitions increase total flame area and decrease the distance each flame front has to cover to complete burn-out. From simple geometrical considerations the 'magnification' of the apparent flame speed resulting from multiple ignitions, separated by distance *x* across a cloud of linear dimension *X*, is approximately $2X/x$ times the normal propagation rate, and this does not take into account additional effects of turbulence. Note that the present hypothesis is not intended to supersede other possible mechanisms which have been postulated. We wish rather to draw attention to a very significant additional effect which must be taken into account if it occurs at all.

A few order-of-magnitude calculations were carried out on the likely rates of temperature rise of particles subjected to such radiation, to check whether the concept was reasonable. Using a mass/irradiated area ratio of 0.02 kg m^{-2} and a specific heat capacity of $1.7 \text{ kJ kg}^{-1} \text{ K}^{-1}$ (typical for cellulosic materials) rates of temperature rise of the order $10^4\text{--}10^5 \text{ K s}^{-1}$ are obtained. Typical auto-ignition temperatures would, therefore, be expected to be reached in $10^{-2}\text{--}10^{-1} \text{ s}$. Moreover, there are likely to be cooperative effects between flammable mixture and heated particle as regards the ignition criterion: the ignition temperature or ignition energy may be considerably lower for a combustible particle in a flammable mixture than that for either a combustible particle in air or for an inert particle in a combustible mixture. It therefore seems reasonable that the available radiation flux could raise the temperature of a particle, irrespective of its size (that is, over dimensions larger than the quenching distance) to a reasonable ignition temperature in the time during which a sufficient radiation source persists.

The short duration of the ignition time lag means that it is the growth of the initial radiation source to a size giving sufficient emissivity that is likely to take the longest time. Thereafter ignition of all the centres irradiated at the same flux may be virtually simultaneous, irrespective of the time lag affecting each

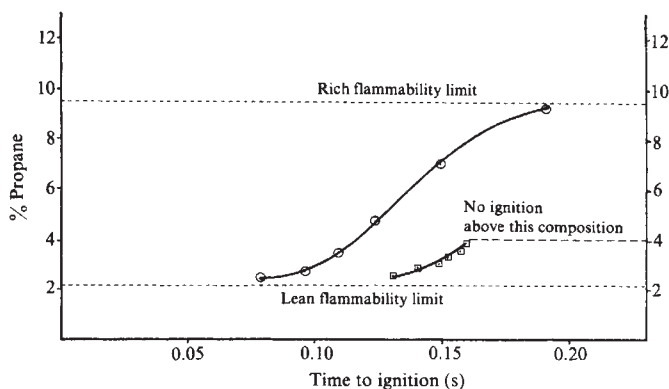


Fig. 2 Effect of gas mixture composition on minimum ignition times (flux = 350 kW m^{-2}) of Kaowool (○) and cotton wool particles (□).

of them. Due to the attenuation of radiation with distance, such initiation will, however, occur progressively ahead of the advancing flame front. The over pressures generated will depend on this rate of progression but the contribution of radiation ignition will not fall to zero until the propagation rate is so fast that the ignition lag, integrated over the range of radiation fluxes ahead of the front, remains incomplete by the time the front reaches the particle.

To study the concept, various particles were irradiated in a flammable atmosphere by a small continuous-wave CO_2 laser constructed in these laboratories with a nominal power of 20 W. The distribution of radiation flux across the beam was determined calorimetrically and peaked at 400 kW m^{-2} . The irradiated particles were suspended on quartz fibres above a burner mouth discharging pre-mixed propane-air in various proportions, metered by rotameters. The time to ignition was measured as a function of radiation flux and mixture strength for each type of particle. The onset of ignition was monitored by means of an ionization probe connected to an oscilloscope. Synchronization of particle irradiation with the start of the time measurement was achieved by placing a steel shutter in the laser beam and connecting this to the trigger input of the oscilloscope. Some results are shown in Figs 1 and 2.

As might be expected the dependence of the results on the form of aggregate of the particles is more complex than simple theory suggests. For compact aggregates such as small pieces of paper, charring occurs without producing ignition in our experimental conditions. However, loose aggregates of fine fibres, such as cotton wool, will ignite in a small fraction of a second at a radiation flux <10% of that corresponding to the black-body radiation at stoichiometric flame temperatures (see Fig. 1) and well within the range of values measured near large fires. Note that non-flammable materials in this form of aggregate, such as the refractory 'Kaowool' produce ignition at least as readily (in fact in less time—see Fig. 2).

Presumably the effectiveness of such particles in producing ignition is due to their low heat capacity for a given volume and area irradiated. The mechanism is now being studied in greater detail. In this context, note that the fibre diameters of Kaowool and cotton wool are $5 \mu\text{m}$ and $20 \mu\text{m}$ respectively. It also seems that the evolution of blanketing vapour from the fibres must be considered in parallel with the rise of temperature in time. Thus it seems likely that the rich limit of ignitable compositions of the mixture is extended to the flammability limit in the case of Kaowool (see Fig. 2) because, unlike cotton wool, it does not evolve any flammable vapour.

The conclusion that materials in this form of aggregate are readily ignited by levels of radiation flux corresponding to those measured in large experimental fires, seems to us of far-reaching importance. Note that the kind of aggregate most conducive to ignition by radiation is also the most likely to be raised by a blast

wave or convection current, due to its low free-fall velocity. If the hypothesis is correct, it suggests that the practical hazard may be alleviated, for example by spraying and thus retaining dangerous dusts on wetted surfaces.

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Decomposing the IR absorption spectra of diamonds

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Nitrogen is the most important impurity found in natural diamonds, affecting properties as diverse as colour¹ and thermal conductivity². Consequently it is important to distinguish between the different molecular forms adopted by the nitrogen. Improved data for obtaining the relative concentrations of two of the most common molecular forms are now presented. These data are then applied to a set of diamonds taken from one source and it is confirmed that the relative concentrations of the different forms of the nitrogen are intimately related to each other.

Most natural diamonds (perhaps 98%)³ contain their nitrogen predominantly in the A molecular form, believed to be a pair of nearest neighbour substitutional atoms⁴, as well as in other aggregated forms. These other forms are the N_3 and N_9 centres which may be identified by UV spectroscopy¹, the B aggregates and the platelets. The platelets produce⁵ a clearly resolved absorption peak at $1,370 \text{ cm}^{-1}$ ($7.3 \mu\text{m}$) as shown in Fig. 1. However, the A and B aggregates give overlapping absorption (Fig. 1). In the spectral region of Fig. 1, pure diamonds are essentially transparent.

To separate the A and B spectra we can take the ratio $m = \mu(7.8)/\mu(8.5)$ of the absorption coefficients measured at $7.8 \mu\text{m}$ ($1,280 \text{ cm}^{-1}$) and at the peak absorption near $8.5 \mu\text{m}$ (near $1,175 \text{ cm}^{-1}$). From m we can obtain the ratio $r = \mu_{7.8}^A/\mu_{7.8}^B$ of the absorption produced separately by the A and B aggregates at $7.8 \mu\text{m}$. Although existing data⁶ relating m and r have been used⁷⁻⁹ these were based on a very few largely unsuitable diamonds and are slightly incorrect when r is large. For the present study diamonds were selected for good homogeneity, with large faces (about $3 \times 5 \text{ mm}^2$) and with optimum absorption strengths (giving minimum transmissions of about 40% of the incident light). It is now found that

$$r = (2.72m - 1)/(1 - 0.41m) \quad \text{when } m < 1.5$$

and

$$r = (1.88m - 1)/(1 - 0.5m) \quad \text{when } m > 1.5$$

(It is preferable to use two expressions, depending on the value of m , as the wavelength of the peak absorption near $8.5 \mu\text{m}$ varies with the A to B admixture, as is obvious from Fig. 1.)

Hence

$$\mu_{7.8}^A = \mu(7.8)r/(1+r); \quad \mu_{7.8}^B = \mu(7.8)/(1+r) \quad (1)$$

where $\mu(7.8)$ is the total absorption measured at $7.8 \mu\text{m}$. An alternative way to obtain r (for example in strongly absorbing diamonds) is to use the absorption ratio m' measured at $9.9 \mu\text{m}$

(1,015 cm⁻¹) and 9.1 μm (1,100 cm⁻¹), $m' = \mu(9.9)/\mu(9.1)$, giving

$$r = (0.795 - 1.164m') / (0.124m' - 0.0333)$$

and

$$\begin{aligned}\mu_{7.8}^A &= \mu(9.1)r / (0.125r + 1.164); \\ \mu_{7.8}^B &= \mu(9.1) / (0.125r + 1.164)\end{aligned}\quad (2)$$

Equations (1) and (2) are believed to give $\mu_{7.8}^A$ and $\mu_{7.8}^B$ correct to $\pm 5\%$ of the total absorption at 7.8 μm.

As an application of these results we consider the relationship obtained by Brozel *et al.*⁷ on the platelet A and B absorption in different diamonds. They worked with specimens taken from arbitrary sources. As the distribution is determined thermodynamically¹⁰ we may obtain cleaner statistics by using specimens from one growth environment. Forty-seven diamonds from an eclogitic environment¹¹ in the Premier Mine (South Africa) gave the relationship shown by the dots in Fig. 2 for the variation of platelet absorption at 7.3 μm divided by $\mu(7.8)$ with the fraction of $\mu(7.8)$ produced by the A aggregates. (All these quantities are measurable directly from the IR spectra.)

For $\mu_{7.8}^A/\mu(7.8) > 0.5$ the data lie on a remarkably uniform trend with three times less scatter than reported by Brozel *et al.*⁷. However, for smaller values of this ratio the data deviate from Brozel's curve⁷ (shown by the line in Fig. 2 after transforming to the axes used here and adjusting the ordinate to allow for the arbitrary scale used in ref. 7). Supplementing the Premier

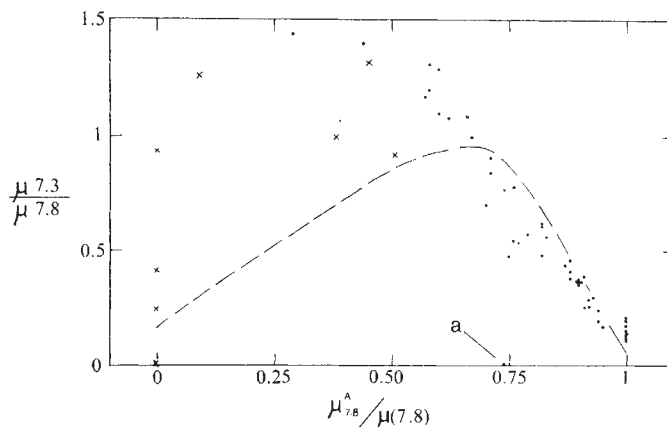


Fig. 2 The variation of (platelet peak absorption divided by total 7.8 μm absorption) with the ratio r of the absorption at 7.8 μm produced separately by the A and B aggregates. Diamond A has a total 7.8 μm absorption of $\mu(7.8) = 0.5 \text{ cm}^{-1}$, far lower than that of most of the other specimens, which had $5 \leq \mu(7.8) \leq 50 \text{ cm}^{-1}$. ●, Diamonds from the Premier Mine; ×, random species. The line is taken from ref. 7 after transformation and scaling.

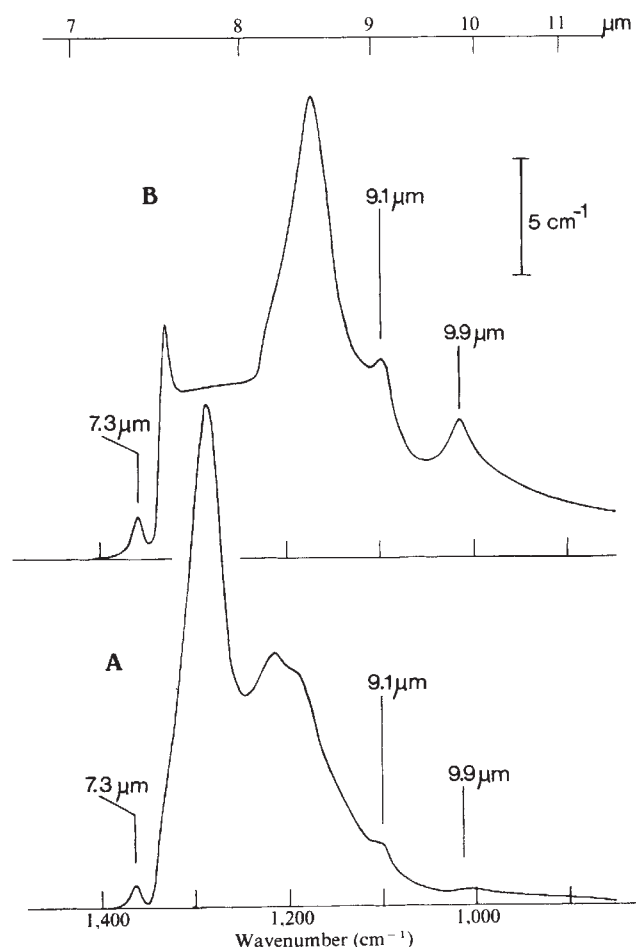


Fig. 1 IR absorption spectra of diamonds containing predominantly A and B aggregates.

diamonds with random specimens produces a scatter and not the unambiguous approach to the origin given by Brozel *et al.*⁷.

The significance of these results is not limited solely to the somewhat esoteric relationships plotted in Fig. 2. It has been shown¹²⁻¹⁴ that single substitutional nitrogen atoms are able to migrate through the diamond lattice at temperatures around 1,800 K, apparently by a vacancy-enhanced diffusion process¹⁴. The immediate end product of the migration is self-trapping to form the A aggregate of nitrogen. Likewise the A aggregates may be broken into single atoms by heat treatment (for example, at 2,250 K)⁷. The equilibrium established by the nitrogen between the single and the paired form depends on the temperatures experienced by the diamonds¹⁰. The other aggregated forms of nitrogen mentioned above also take part in the equilibration process^{9,10}. Viewed in this context, the relationships shown in Fig. 2 suggest that, in principle, the relative concentrations of nitrogen in a given diamond can be used to estimate the temperatures experienced by that diamond since its formation; the conditions prevailing at its formation can be determined from the nature of the inclusions trapped within it at the time of growth¹¹. As well as being of interest to the geologist, this approach is important to the gem industry because the restructuring of the nitrogen impurity by heat treatment can improve the colour of a diamond¹⁷.

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Quantitative retention of magmatic argon in a glassy basalt

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Basalts erupted in deep waters commonly chill sufficiently rapidly to form a glass outer skin. Due to the rapid chilling and the high hydrostatic pressures, magma-ambient gases and in particular the rare gases may be trapped within this glass¹⁻³. Several theoretical investigations have examined the relationship of the trapped $^{40}\text{Ar}/^{36}\text{Ar}$ ratio to the history of terrestrial degassing and formation of the atmosphere⁴⁻¹¹. These measurements have not yet, however, answered the fundamental questions of whether the mantle $^{40}\text{Ar}/^{36}\text{Ar}$ ratio varies significantly either vertically within the Earth and/or from one geographic locality to another, or even whether a quantitative estimate of the magmatic concentration and isotopic ratio at any given location is in fact obtainable. This report deals with the last of these questions. Our data indicate that the answer is in the affirmative.

In any sample, the Ar actually measured will consist of unknown proportions of a trapped magmatic and a contaminating atmospheric component, and it is possible that the trapped magmatic component may not be quantitatively retained in the

Table 1 Ar data measured in seamount P-50 (5°59'S, 98°09'W)

Sample no.	Description	^{36}Ar ($10^{-10}\text{ cm}^3\text{ g}^{-1}$)	$^{40}\text{Ar}/^{36}\text{Ar}$ ($10^{-10}\text{ cm}^3\text{ g}^{-1}$)
1	0.68 g glass; mildly heated	0.3 ± 0.1	275
2	Above sample melted	2.05	8,370
3	1 + 2	2.35	7,366
4	0.4 g glass; mildly heated	0.99	295
5	Above sample melted	1.54	11,300
6	4 + 5	2.53	6,960
7	1.2 g glass; mildly heated	1.1	455
8	Above sample melted	1.85	8,890
9	7 + 8	2.95	5,760
10	Interior rock	4.3	1,570
11	Interior rock	8.4	1,880

glass. In what follows, $^{40}\text{Ar}_a$ and $^{40}\text{Ar}_m$ denote atmospheric and the unknown mantle ^{40}Ar , while m and a denote their ratios ($^{40}\text{Ar}_m/^{36}\text{Ar}_m$) and ($^{40}\text{Ar}_a/^{36}\text{Ar}_a$) respectively, the former being, of course, unknown. Then¹²

$$\begin{aligned} \left(\frac{^{40}\text{Ar}}{^{36}\text{Ar}}\right)_{\text{measured}} &= \frac{^{40}\text{Ar}_m + ^{40}\text{Ar}_a}{^{36}\text{Ar}_{\text{measured}}} \\ &= \frac{m^{36}\text{Ar}_m + a(^{36}\text{Ar}_{\text{measured}} - ^{36}\text{Ar}_m)}{^{36}\text{Ar}_{\text{measured}}} \\ &= \frac{(m-a)^{36}\text{Ar}_m}{^{36}\text{Ar}_{\text{measured}}} + a \end{aligned} \quad (1)$$

If, therefore, the measured $^{40}\text{Ar}/^{36}\text{Ar}$ plotted against $(^{36}\text{Ar})^{-1}$ gives a straight line for a suite of samples, $(m-a)^{36}\text{Ar}_m$ must be constant for those samples and the concentration and/or isotopic ratio of the trapped component may then be obtainable. Of two early sets of data, one^{1,13,14} gave two reasonably straight lines while the other¹⁵ showed only random scatter. A later set of data from a DSDP core gave an excellent straight-line fit¹⁶. These data are summarized in Fig. 1. Recently Dymond *et al.*¹⁷ combined these data with their own unpublished data and concluded that they show merely random scatter below the upper-limit envelope (Fig. 1a); such behaviour would indicate post-eruptive diffusive loss from the glassy basalts and limit the retrievable information to only a lower limit of the product $(m-a)^{36}\text{Ar}_m$.

It is important to determine whether samples exist which have quantitatively trapped the magmatic Ar without such subsequent diffusive loss, so that reliable estimates of the concentration and isotopic ratio may be determinable in principle and so that the search for vertical and/or lateral differences may be profitably pursued. These data on several determinations of Ar concentrations from one dredge haul, seamount P-50 from the south-east Pacific, (previously described¹) fit the straight-line relationship of equation (1) and thus indicate quantitative retention of the trapped Ar. It is important that this contention refers to the magmatic gases present in the lava as it erupts onto the surface of the Earth. Processes of elemental fractionation¹⁸ or vesiculation¹⁹ preceding eruption might enhance or deplete the elemental concentrations in the glass phase, but would not affect the isotopic ratios.

The data of Table 1 were obtained using a stainless-steel Reynolds-type rare gas mass spectrometer by a process similar to that described in ref. 20. The samples were chips taken from the glass crust of a single basalt flow. Blank levels were $\sim 2 \times 10^{-11}\text{ cm}^3$ for ^{36}Ar ; accuracy, $\pm 10\%$; precision of the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio, $\sim 2\%$. Samples 1, 4 and 7 were heated for 30 s, sufficient to raise the crucible to a red glow, in an attempt to separate the (surface-adsorbed) atmospheric from the internally trapped components. The technique was at least partially successful:

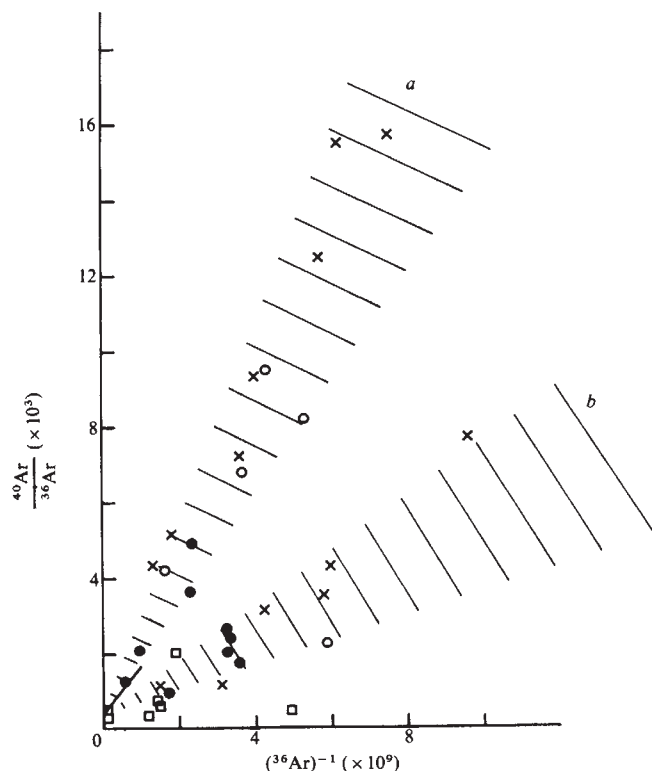


Fig. 1 $(^{40}\text{Ar}/^{36}\text{Ar})_{\text{measured}}$ plotted against $(^{36}\text{Ar})^{-1}$. The data of refs 1, 13 and 14 were measured on glasses from the East Pacific Rise and the Vema Fracture Zone. The solid line shows the slope and extent of samples from a DSDP Cretaceous core taken from the southern end of the Bermuda Rise. ●, Ref. 1; ○, ref. 13; ×, ref. 14; □, ref. 15.

runs 1 and 4 show only atmospheric Ar, while run 7 contains a small amount of magmatic Ar, identified by the higher $^{40}\text{Ar}/^{36}\text{Ar}$ ratio. None of the samples 1, 4 and 7 would be expected to fall on the 'constant-magmatic' line, as they obviously cannot reflect a constant-magmatic Ar component; indeed, they all lie far off the curve, to the lower right of Fig. 2. As only zero or insignificant amounts of magmatic Ar were removed from the samples in these runs, both the high-temperature fractions and the total abundances should plot along the constant-magmatic line, as, in fact, they do. Samples 10, 11, 18 and 19 are interior rock specimens of P-50; such interior rock are expected not to retain quantitatively the magmatic gases²¹, and indeed these samples lie below the constant-magmatic line.

Figure 2 includes previously published data from this same seamount; the total data bank thus includes measurements on several different samples of this dredge haul, made with three different mass spectrometers at two different laboratories over 12 yr. The straight-line fit to the glass samples (shown in Fig. 2) is remarkably good over the entire range of measurements ($^{40}\text{Ar}/^{36}\text{Ar} = 1,282\text{--}11,300$). Anchored at an intercept of 295,

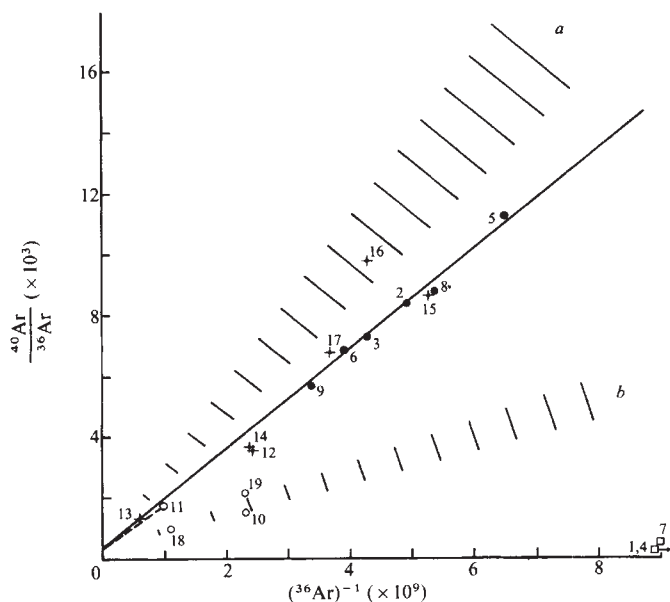


Fig. 2 The envelopes *a* and *b* are from Fig. 1. Samples 1–11 are from Table 1. Sample 12 is from ref. 14; samples 13 and 14 from ref. 1; samples 15–19 from ref. 13. ●, Glasses, this work; +, glasses, previous work; ○, interior rock; □, first (mild) heating.

an accurate world-wide average of the $^{36}\text{Ar}_m$ component alone, as is emphasized by Table 1, in which many of the samples show significantly smaller concentrations of total measured ^{36}Ar . I prefer to rewrite equation (1) as:

$$(^{40}\text{Ar}/^{36}\text{Ar})_{\text{measured}} = \frac{(m-a)}{m} \cdot \frac{^{40}\text{Ar}_m}{^{36}\text{Ar}_{\text{measured}}} + a \quad (2)$$

and then recognize that the maximum measured $^{40}\text{Ar}/^{36}\text{Ar}$ datum is necessarily a lower limit to *m*, so that from sample 5 $m \geq 11,000$, that is $m \gg a$ and $(m-a) \sim m$. The slope of the line then gives the $^{40}\text{Ar}_m$ concentration directly: $172 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$. With $m \geq 11,000$, the concentration of $^{36}\text{Ar}_m \leq 1.5 \times 10^{-10} \text{ cm}^3 \text{ g}^{-1}$.

For the Leg 51 DSDP data the analysis is not as straightforward. We can say that the $^{40}\text{Ar}_m$ must be $\geq 143 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$; if the $^{36}\text{Ar}_m \leq 1.5 \times 10^{-10}$, then $m \geq 9,500$. On the other hand, the limit to *m* set by these data alone is $m \geq 1,700$, and then $^{40}\text{Ar}_m \leq 173 \times 10^{-8} \text{ cm}^3 \text{ g}^{-1}$ and $^{36}\text{Ar}_m \leq 10 \times 10^{-10}$. The mantle component therefore looks similar, but not necessarily identical, to that recorded in the P-50 glass. The similarity in both concentration and isotopic ratio allowed by the data for Ar erupted on the other side of the world and 100 Myr previously is striking.

The data from seamount P-50 show that: (1) Samples do exist which have trapped the magma-ambient rare gas component without post-eruptive diffusive loss. (2) In the region sampled by the P-50 magma, the mantle ratio $^{40}\text{Ar}/^{36}\text{Ar}$ is $\geq 11,000$, more than an order of magnitude higher than several other estimates of the mantle ratio^{4,10,23,24}. (3) Any one measurement of the amount and isotopic ratio of Ar in a given sample is not quantitatively significant, as atmospheric contamination is generally present. Also, unless a straight-line relationship among several samples can be demonstrated, diffusive loss of the mantle component cannot be discounted.

If further measurements show this straight-line relationship in suites of samples erupted from varying depths and geographic positions, and in varying tectonic conditions, we should be able to determine whether or not a reasonably unique worldwide mantle component exists, and thus whether models of atmospheric evolution and terrestrial degassing based on such mantle-wide parameters have a chance of being valid. Alternatively we may be able to document any vertical and/or lateral variations in this component, and thus provide information pertinent to models which require such variations¹⁷.

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corresponding to the model relationship

$$(^{40}\text{Ar}/^{36}\text{Ar})_{\text{measured}} = (m-a) ^{36}\text{Ar}_m / ^{36}\text{Ar}_{\text{measured}} + a$$

the excellent straight-line fit indicates a constant value for $(m-a) ^{36}\text{Ar}_m$. In this equation both *m* and $^{36}\text{Ar}_m$ are unknown. Figure 1 shows that a similar relationship was found by Takaoka and Nagao for DSDP samples from Hole 417D of Leg 51 (Cretaceous basalt flows in the Caribbean¹⁶). They solved equation (1) for *m* by assuming that either all the ^{36}Ar they measured was trapped magmatic, or alternatively that $^{36}\text{Ar}_m = 5 \times 10^{-10} \text{ cm}^3 \text{ g}^{-1}$ in all the samples. The first assumption provides only an upper limit to $^{36}\text{Ar}_m$; it is obviously an overstatement, as it denies the existence of the constant-magmatic line demonstrated by their data. The second assumption is based on Craig and Lupton's²¹ analysis of the early data^{14,15,22}, which include both atmospheric and magmatic Ar with no attempt to differentiate between the two; the result being no more than an average of all total measured values. This cannot possibly reflect

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ESR dating of ancient flints

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Natural radioactivity can produce measurable amounts of radiation damage in rocks and glasses, and the gradual accumulation of this damage with time provides a built in 'clock' that can be used as a dating tool for geology and archaeology. Although a studied technique for estimating this damage involves measurements of stored thermoluminescent (TL) activity¹⁻⁴, several authors have reported the direct observation of natural radiation damage by ESR⁵⁻⁷. We describe here ESR measurements on a damage signal which we have found to be present without exception in a wide variety of natural flints. The point defect responsible for the signal is identified and the samples are calibrated with an artificial damage simulation. Preliminary age determinations are presented for several natural flints. Flint artefacts heated in antiquity will have their clock 'reset' to zero, and measurements on one sample suggest that the ESR technique may have applications in the study of early cultures.

Flint is a hydrothermally grown microcrystalline silica rock. Typical samples contain α quartz, amorphous silica, various silica allomorphs and a few per cent of non-silica 'binder' material. The ESR spectrum generated by natural radioactivity is shown in Fig. 1a. This spectrum is very similar to that found in quartzes artificially damaged by fast neutron bombardment⁸,

damage signal shown in Fig. 1d has two major components; the sharp structure between $g = 2.000$ and $g = 2.0020$ associated with a dangling Si bond (an E' centre), and a broad but well defined, $S = 1/2$, powder pattern at lower field. Ignoring the E', we find the low field signal grows smoothly and uniformly with fast neutron damage, and thermally anneals away as a unit. Pure γ -ray radiation (^{60}Co , up to 3 MR) has little effect on this signal in natural flints. It gives no measurable increment to the natural signal, and in samples where the natural signal has been annealed away, the weak low field signal produced by γ rays has a distinctly different appearance. (An interesting exception is when the natural signal is only partially annealed away, when even a small γ -ray dose will reconstitute a fraction of the original signal before saturating. Presumably, structural precursors are being repopulated here.) An examination of the g values of the natural signal suggests an interpretation for this insensitivity to γ rays. The values agree well with those of the recently identified 'peroxy centre'¹¹, an O_2^- radical bonded at one end to a silicon atom. If we accept this identification, then the defect is an interstitial oxygen, and a displacive lattice collision is needed to produce it.

Whether the structural damage is due to α -recoils or fast neutrons, the ultimate source of point defects is a cascade of displacement collisions in the wake of an energetic (kiloelectron volts) oxygen or silicon ion (a primary knock-on or PKO), and to first order the only parameter required for calibration is the energy deposited in the lattice. For the natural damage this is a straightforward computation. Each emission leads to a recoil nucleus with some 80 keV of energy that ultimately finds its way to the lattice through PKOs. Summing over all decays (^{238}U , ^{232}Th and their daughters in secular equilibrium) leads to an energy deposition rate

$$P_r = 8.12 \times 10^5 (\text{MeV yr}^{-1} \text{ cm}^{-3}) \times C_{\text{eff}} \quad (1)$$

where C_{eff} is the effective radioactive impurity concentration in $\mu\text{g per g}$, given by $C_{\text{eff}} = C_{\text{U}} + 1/3 C_{\text{Th}}$.

Table 1 ESR ages of some geological flints

Sample	Original flint	Accepted geological age (Myr)	t (s)	T		P		ESR age (Myr) $\pm 30\%$
				C_{U} ($\mu\text{g per g}$)	C_{Th} ($\mu\text{g per g}$)	C_{U} ($\mu\text{g per g}$)	C_{eff} ($\mu\text{g per g}$)	
3 g	Oklahoma	370	1,507	2.25	3.07	0.26	0.67	310
5 g	Utah	250	1,854	3.00	0.415	0.41	0.70	366
7 g	Africa	3,200(?)	304	0.693	0.802	0.52	0.74	57
9 g	Missouri	320	700	1.11	0.209	0.18	0.29	333
Ill	Illinois	320	1,347	1.20	1.20	0.42	0.664	280

Column 4 gives the average reactor exposure needed to regenerate the native ESR signal, with all data scaled to row 1, site H, reactor conditions. The effective radioactive concentration in column 8 is computed from the measurements of total concentration and powder only concentration in the preceding three columns, as described in the text. The uncertainty quoted in the final column includes statistics and a rough estimate of possible systematic errors.

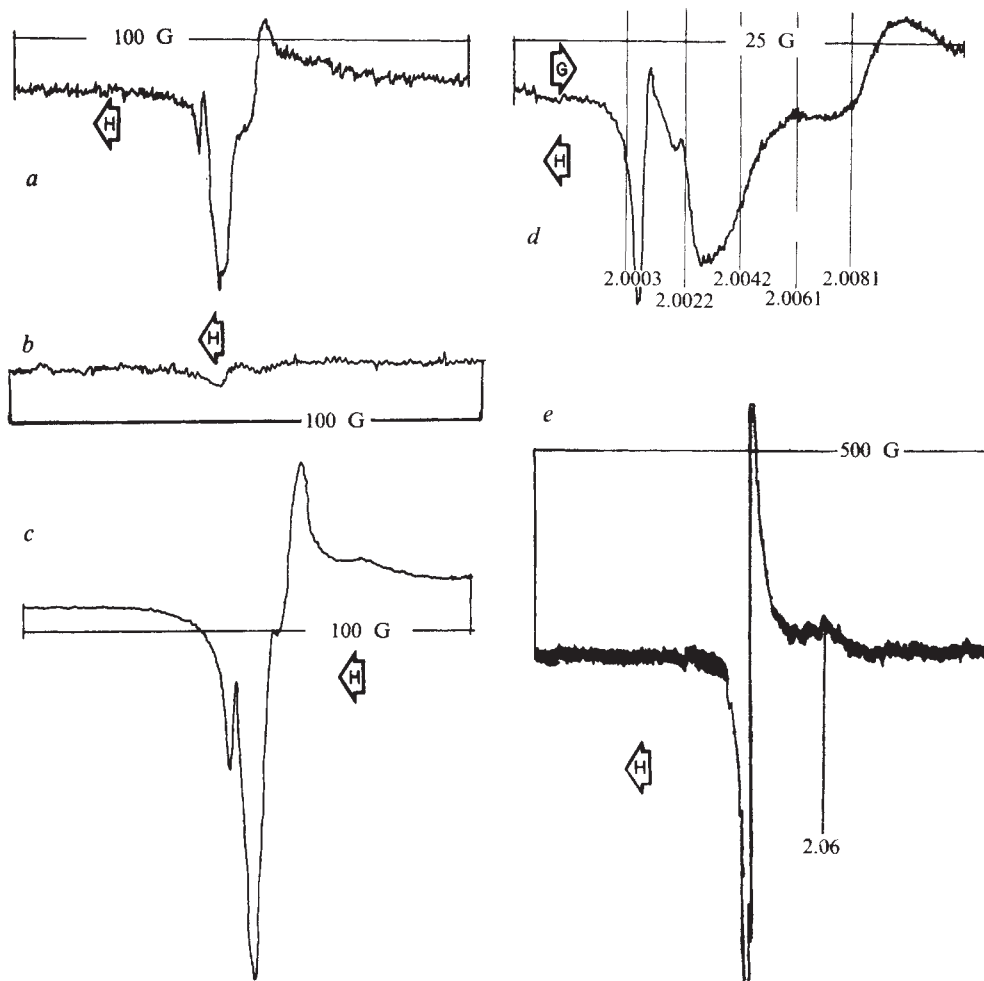
and also to the native signal observed by McMorris⁵ in igneous (but not in hydrothermal) quartz minerals. We believe, with McMorris, that this signal appears because of structural damage in the silica lattice due to the momentum-conserving nuclear recoils of α -emitting impurities. (β , γ and even α particles dissipate nearly all their energy in electronic processes and do little structural damage.) The signal thermally bleaches at 500 °C (Fig. 1b) and is readily regrown by neutron damage (Fig. 1c).

Recent work on radiation-damaged silica^{9,10} has led to a fairly clear picture of some of the simpler point defects in the silica lattice. The expanded, high-resolution trace of the natural

The total ^{238}U and ^{232}Th concentrations were determined directly by neutron activation analysis. Because flint is heterogeneous, we need to correct these measurements for the fraction of radioactivity in the binder instead of the silica lattice. We estimate this partitioning by crushing the samples and etching them in hot aqua regia. This procedure leaves a fine white powder of silica microcrystals, but removes the binder. ESR of the powder shows that etching removes impurity signals (particularly Mn^{2+}) but leaves the original peroxy signal only slightly reduced. The powder is then re-assayed for ^{238}U and we assume the less important ^{232}Th is distributed the same way. It is probably wrong to neglect the radioactivity in the binder entirely, because some of these atoms will be registered on the crystal interfaces where they can still damage the lattice (the recoil range is $\sim 200 \text{ \AA}$), so we include the binder fraction in our estimates of C_{eff} , but weighted with an efficiency of 0.1.

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Fig. 1 Absorption derivative ESR signals in Illinois flints. $\nu = 9.1$ GHz, $H_0 = 3,200$ G. *a*, The natural signal, $T = 300$ K. *b*, The same sample after annealing at 773 K. *c*, The same sample after exposure to radiation damage by fast neutrons. Correcting for gain and modulation, the signal is 12 times larger than the natural signal in *a*. *d*, An expanded high-resolution trace near $g = 2$, showing powder pattern turning points near $g_1 = 2.002$ and $g_2 = 2.008$. *e*, A high-gain, high-modulation trace at $T = 100$ K, showing the powder pattern turning point near $g_3 = 2.06$.



To calculate the energy deposited by fast neutrons, the neutron flux distribution must be evaluated at the irradiation site. We have done this by combining reactor-design calculations and measuring thermal neutron fluxes. (In the Missouri University Research Reactor, 27.8 cm from the centre of the core row 1, site H, we find $(d\phi/dE)dE = (k/E)dE$, $E < 3$ MeV, with $K = 4.5 \times 10^{12}$ neutrons per $\text{cm}^2 \text{s}^{-1}$.) These relatively low-energy collisions are nearly isotropic and the total energy given to PKOs is readily computed¹². However, the PKO spectrum has a significant high-energy component, so some of this energy is lost in irrelevant electronic processes. Evaluating this effect with a standard model^{13,14}, we find an overall efficiency of 0.36 for producing structural damage, and an energy deposit rate in the lattice (in row 1, site H of our reactor) of

$$P_n = 1.12 \times 10^{11} \text{ MeV cm}^{-2} \text{ s}^{-1} \quad (2)$$

Combining equations (1) and (2) then leads to

$$\text{Age (Myr)} = 0.139t(\text{s}) / C_{\text{eff}}(\mu\text{g per g}) \quad (3)$$

where t is the reactor exposure time needed to reproduce the native signal.

This last expression was used in a blind study of several old flints. The results are presented in Table 1, along with the accepted geological ages. Except for the Precambrian African sample (an unusual flint subjected to obscure geological processing), the agreement in ages seems very satisfactory.

As expected, flints known to have been heated in the past (by reduced TL) showed a marked reduction in the peroxy signal. One heated sample was investigated more carefully. It came from Combe Grenal France, in an Acheulian archaeological stratum deposited $\sim 2.5 \times 10^5$ yr BP (ref. 15). The ESR spectrum near $g = 2.00$ is contaminated by impurities, but the long plateau

region extending towards $g = 2.06$ (Fig. 1*e*) shows clear evidence for an elevated absorption when compared with a sample heated in the laboratory. The characteristic peroxy signal reappears after neutron bombardment, and a (lengthy) extrapolation suggests that the reactor exposure time needed to reproduce the original signal would be about 5 s. The total ^{238}U concentration is $2.5 \mu\text{g per g}$, and if this U is partitioned normally between the silica and binder then the maximum elapsed time since heating would be about 5×10^5 yr. Because some of the plateau signal observed could come from centres other than the peroxy, or from an unannealed residue, the heating could well have been more recent. The main refinement needed for dating young samples is not so much an improved signal-to-noise ratio, but rather an ability to discriminate fresh peroxy signal from interfering background. This refinement may prove to be within the capabilities of standard ESR techniques.

The sample from Combe Grenal France was obtained from Professor Francois Bordes.

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Stones, pits and Stonehenge

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There have been many mathematical or astronomical explanations of the construction of certain elements at Stonehenge¹⁻⁹. In 1979 a small salvage excavation at the site resulted in the chance discovery of a prehistoric pit dug to take a standing stone. The recognition that such substantial structures can exist unknown at Stonehenge is important to those who seek alignments or patterns in the locations of neolithic features. That 'known' arrangements could be affected by such unexpected discoveries is here illustrated with a preliminary reassessment of the significance of the Heelstone and the Station Stones (a full description of all aspects of Stonehenge is given in ref. 10).

In May-June 1979, a trench 24×1.5 m was excavated along the proposed route of a telephone exchange cable on the roadside verge (Figs 1, 2). Besides the expected segments of the circular ditch around the Heelstone and the eastern ditch of the Avenue, a completely unexpected prehistoric feature was found. This comprised part of a wide pit with the impression on its base of the stone it had once held upright. No direct dating evidence was obtained, but the pit apparently relates to early activity at the site. The standing stone was removed, and the pit backfilled and fully consolidated before the ditch around the



Fig. 2 Excavation in progress, looking south-east. The stone pit shows as a large depression in the chalk, truncated by the Heelstone ditch which crosses the trench in the foreground. Emerging from unexcavated ground to the right, is the smooth, crushed chalk surface created by the weight of the standing stone. The rectangular excavation at the bottom of the photograph shows the point reached by the Post Office cable when archaeological investigation began. Behind the coils of barbed wire (erected around the site for the period of the summer solstice festivities) can be seen the base of the Heelstone.

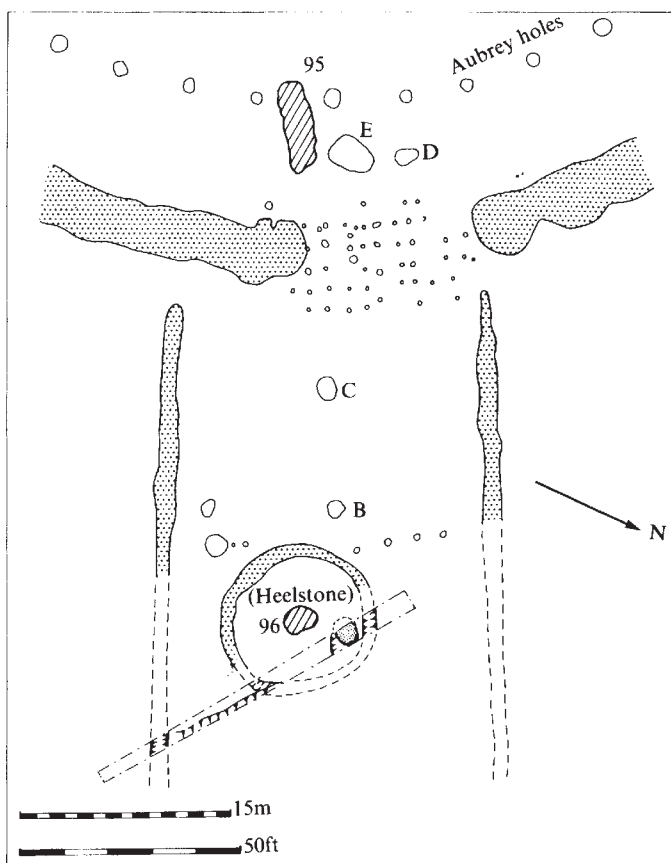


Fig. 1 Area around the entrance to the earthwork enclosure at Stonehenge, showing pits (letters), stones (numbers) and the trench excavated in 1979. The newly discovered pit is north of the Heelstone, and the stone impression is shown shaded.

Heelstone was excavated; this ditch had been refilled at the time the ditches and banks defining the Avenue were created, around 2170 BC (BM-1164: 1728 ± 68 b.c., HAR-2013: 1770 ± 70 b.c.; calibrated after Clark¹¹, 2140 ± 150 BC and 2200 ± 155 BC respectively).

The location of this previously unknown stone (called stone 97 in the sequence, after number 96, the Heelstone) questions the relationship of the Heelstone to the site's alignment. The eccentric position of the latter stone to the main axis of the site, which is approximately oriented on the midsummer sunrise has proved puzzling. If stone 97 was standing at the same time as the Heelstone, and the two were erected as part of a single plan, the problem may be solved, for the two stones sit astride the axis. Indeed, Newall predicted the presence of a stone beside the Heelstone on this hypothesis¹². The midsummer Sun would then rise on an alignment, accidentally or deliberately contrived, between the two stones. However, the Heelstone may have been erected after the now missing stone had been removed, with or without a period during which no stone was there at all. This interpretation was favoured by Atkinson (personal communication) who calculated the azimuth of the apparent centre of the impression left by stone 97 from the estimated centre of Stonehenge I as about $48^\circ 21'$. The precise location of the stone could not be determined from the excavated evidence—the stone's shape and size above ground are only definable within very broad limits.

Adopting the hypothesis of the Heelstone as one of a pair of stones, how does this pair fit into the overall arrangement of known stone locations at the site? The distance¹³ both between the main sarsen circle (specifically stones 1 and 30) and the pair of stones at the earthwork entrance (stone 95 and that represented by hole E), and the distance between this last pair and the Heelstone is some 30 m. Can we extend this pattern and interpolate an "imaginarie Walke of stones" similar to those at Avebury¹⁴?

The confused picture presented by antiquarian literature^{15,16} has prompted magnetic and resistivity surveys along part of the Avenue. The results show a strong disturbance in the chalk precisely where the next pair of stones should be, moving on from the Heelstone. The geophysical anomaly indicates the possible presence of only one pit. However, the model would predict this, because for both the known pairs the northwestern stone was removed in prehistory and the pit backfilled with chalk: it cannot be assumed that either of these pits would be visible to sub-surface detecting devices.

The interpretation of the newly discovered stone 97 as a partner for the Heelstone does not, however, exhaust all possibilities; Atkinson has other opinions. An alternative would be that the stone is part of a single row, of which two other stones were identified by Hawley. The now vacant pits (labelled by Atkinson as B and C) and the new pit are equidistantly spaced on a straight alignment. The apparently smaller size of holes B and C, however, suggests that the stones they contained (and note that the existence of these stones is largely hypothetical) did not match the dimensions of 97. Indeed, the new pit is so large, apparently having been sectioned by Atkinson *et al.* in 1956 (ref. 17), that it possibly consists of intersecting pits that fulfill both these suggested roles.

Four outlying features, in addition to the Heelstone, are frequently used in complex geometrical or astronomical constructions. Of these four 'Station Stones', only two survive in position; the others are evidenced by poorly described hollows in the chalk. Their importance lies in their location on the four corners of a perfect rectangle that is aligned on the site's major axis. However, evidence suggests both that the Heelstone and the Station Stones may have had companions, and that the basis of the latter's location was not so much a rectangle as a circle. For this interpretation, we must consider a ring that lies on the periphery of the site.

The 56 more or less regularly spaced pits in this ring were first known as the 'X holes' and later as the 'Aubrey holes'¹⁸, although the association of these 56 pits with the five 'cavities' observed by Aubrey is unconvincing, not least because of the discrepancy in numbers. Note also that these five hollows, supposedly representing the collapsed fill of pits levelled well over four millennia before Aubrey's visit, were apparently

invisible when Gale and Stukely were at Stonehenge less than 60 years later, to Cunnington and Hoare at the end of the eighteenth century, and certainly not to be seen by Hawley or Newall in this century. It therefore seems likely that if the cavities really were prehistoric in origin, whatever they contained had been removed not very long before Aubrey's visit. This being so, they could only have held standing stones.

Atkinson considered the possibility that the Station Stones were the remnants of a former circle, but has preferred to associate them with the large sarsen structure as four outliers, reserving "the bare possibility that they once formed part of an earlier setting of sarsens within the bank"¹⁹. The main evidence for this putative circle consists of three hollows on the site's perimeter—pits F, G and H. As Atkinson and his colleagues noticed, these pits are correctly located to form, together with the Station Stones and 13 hypothetical pits (mostly in unexcavated parts of the site), a circle of perhaps 20 more or less evenly spaced pits, two of which still retain stones, just outside the ring of X holes. A search was made for one of these pits in the 1950s (in the region of X holes 4 and 5) with a variety of sub-surface detecting devices, but nothing was found²⁰. However, it is possible that a pit does exist, but with a chalk fill rendering it effectively invisible without excavation (as would probably have been the case with the newly discovered pit). Hawley considered at least two of these hollows to be natural (one became known as the 'bush hole'), but this appearance could be given by pits with chalky fill exposed by an excavator with very limited knowledge of pedology and the complex geomorphology of the site. Finally, there was circumstantial evidence²¹ that a stone existed on the perimeter of this hypothetical circle near X hole 28.

The cavities which Aubrey observed exhibit both a coincidence in number with the above features, and a remarkable similarity in their respective locations (Fig. 3). The cavities match holes F to H, and Aubrey's two stones the Station Stones 91 and 93. The remaining cavities would correspond to holes now conventionally described as pits for Station Stones 92 and 94. The only major discrepancy lies in Aubrey's relative transposition of stone 93 and the hollow for 'stone' 94. Without further excavation, the existence of this stone circle must clearly remain an open question.

This consideration of the Station Stones and the Aubrey holes, like the description of the newly discovered stone 97, exemplifies an unavoidable, if inconvenient, problem concerning Stonehenge—we really know very little indeed of the nature and sequence of activities and structures at the site.

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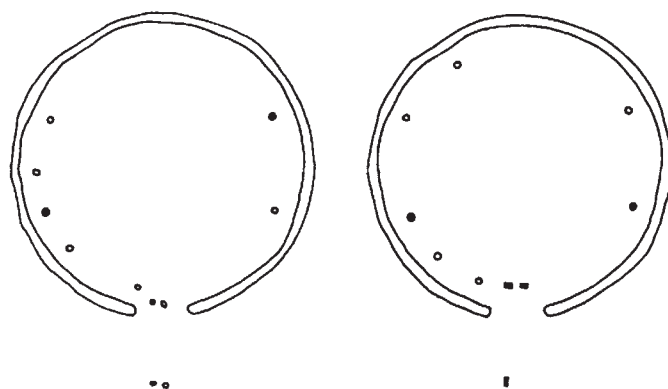


Fig. 3 An outer stone circle? Left: Holes F, G, H, 92 and 94 (○, working clockwise from the enclosure entrance) and the two Station Stones 91 and 93 (●); also shown are the locations of stone 95 and hole E (on the causeway) and stone 96 and the pit for stone 97 (outside the enclosure ditch). Right: the 'cavities' (○) and stones mapped by Aubrey. Enclosure ditch shown schematically.

Disappearance of common skate *Raia batis* from Irish Sea

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Records show that the common skate, *Raia batis*, has declined in abundance in the Irish Sea since the early years of the twentieth century, and is now very rare. As I report here, it is possible to calculate the highest mortality which the species will withstand without collapsing. It is likely that the mortality due to fishing has exceeded this level for some time and that the species will not recover while fishing continues. This represents the first clear case of a fish brought to the brink of extinction by commercial fishing.

In 1902 Herdman and Dawson¹ wrote that the common skate, *Raia batis*, was: "abundant in all parts (of the Irish Sea), and taken by line and by trawling all year round on nearly all our fishing grounds. The young are frequently taken in shrimp nets in shallow water". But the records of more than 800 trawl catches made by research vessels of the Ministry of Agriculture, Fisheries and Food (MAFF) throughout the Irish Sea during the past 10 yr show that no specimen of that fish was taken. None has appeared in trawl catches during research cruises by the Marine Science Laboratories, Menai Bridge during the past 15 yr (E. I. S. Rees, personal communication).

Enquiries among fishermen working in all parts of the Irish Sea indicate that *R. batis* was not uncommon in the late 1940s and that a few were taken during the 1950s and early 1960s, but there have been no recent records. In 1960, E. Hardy published a note in *Fishing News* on "The monster skate of Caernarvon Bay", giving details of a 9-foot-long specimen taken in September of that year. The Irish Specimen Fish Committee publishes an annual report, which in 1976 gave details of specimen-sized skate (more than 120 pounds) taken by anglers in Irish waters since 1956. Of those 20 specimen fish, 15 were from Strangford Lough. However, because of the decline in the skate populations, the Committee has suspended the species from its lists since 1976.

The identification of skate and ray species in the commercial landings in England and Wales is incomplete and they are not always recorded separately, however during the past 5 yr an average of less than 10 specimens of *R. batis* from the Irish Sea has been recorded annually at Fleetwood and these have all been from the North Channel. French commercial landings of *R. batis* from the area west of Scotland and Ireland are much higher, but here too the decline has been very rapid. Trawlers from Concarneau landed 922 tonnes of *R. batis* from this area in 1969, but only 85 tonnes in 1979. During the same period the landings of all other species of rays at Concarneau fell by only 15% (M. H. Du Buit, personal communication).

Several aspects of the skate's biology make it particularly difficult to protect from overfishing. Du Buit², commenting on the declining catches by French trawlers in the Celtic Sea, ascribed its vulnerability to slow growth, high age of maturity and low fecundity. Holden³ has modelled the population dynamics of other ray species from the Irish Sea, but did not distinguish between the mortality on immature and mature fish, and thus did not identify the crucial importance of fishing mortality on immature fish in determining the level of exploitation at which the stock would collapse. I have adapted Holden's method and have used his rates of egg laying to calculate the levels of mortality on immature and mature fish beyond which the stock will collapse.

For a population to remain in equilibrium, the mortality rate of mature fish (Z_m) must equal the net rate of recruitment of mature fish. The net recruitment is given by the number of eggs laid per female per year ($E/2$, because only eggs developing into

females are included) multiplied by the survival from egg laying to maturity ($e^{-Z_i t_m}$, if the immature mortality Z_i occurs evenly over the number of years t_m needed to reach maturity).

$$Z_m = (E/2)x e^{-Z_i t_m}.$$

The number of eggs laid per female depends on the size of the female and hence will vary because the average size of mature fish is affected by changing Z_m . For ray species, Holden⁴ has shown that the rate of egg laying is proportional to body length, not weight, so that there will be little change in $E/2$ as Z_m varies. The rate of egg laying by *R. batis* is not known but can be estimated as about 40 per year, using a relationship between fecundity and size at birth⁵. According to Du Buit², the age of first maturity for *R. batis* is 11 yr. The equation thus gives the relationship between Z_m and Z_i shown in Fig. 1. Any combination of values of Z_m and Z_i to the right of curve *a* will result in the collapse of the population. If the population was subject only to instantaneous natural mortality of say 0.2 before maturity, then a very high rate of exploitation would be possible on the mature stock, although this of course assumes that the direct relationship between number of mature females and net recruitment persists at all stock levels, which is unlikely. However, the fishing mortality on immature fish is probably the same as that on mature fish, because they are vulnerable to capture from birth by the same gear.

R. batis hatch from the egg at a length of ~22 cm, with a disk width of 13–15 cm and are retained by the mesh sizes in use. The line expressing the ratio $Z_m = Z_i$ is shown in Fig. 1 and it can be seen that the stock will collapse at a mortality above ~0.37. Even if the immature mortality were only half of the mature rate (that is $Z_m = 2 \times Z_i$, also shown in Fig. 1) the stock would still collapse when Z_i was 0.31. In fact, the immature mortality may well be higher than the mature mortality, because in addition to being caught in the same gear as the mature fish, the young skate were also taken frequently in shrimp nets in shallow water. Also, no account has been taken of egg and hatching mortality, which may be high and will act in a manner exactly analogous to reduced fecundity. Curve *b* in Fig. 1 expresses either 50% egg mortality or fecundity of only 10 female eggs per year instead of 20. This shows that reduced (or increased) fecundity has a relatively small effect on the mortality at which the stock collapses. It is the net rate of recruitment which is important, and this is determined largely by the cumulative mortality during the immature stage.

No estimates of mortality rates are available for the ray species of the Irish Sea, but they are likely to be of the same order as those on other demersal species, which range from ~0.4

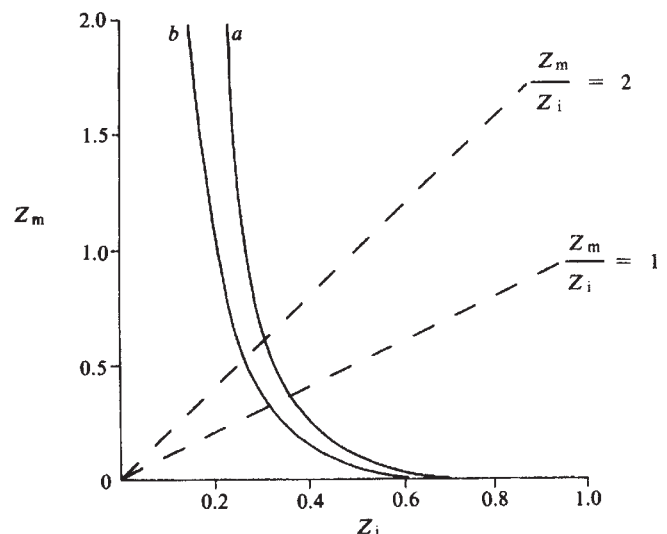


Fig. 1 Values of Z_m (mortality rate on mature fish) and Z_i (mortality rate on immature fish) to maintain the population in equilibrium. *a*, Fecundity = 20♀ eggs per yr; *b*, fecundity = 10♀ eggs per yr.

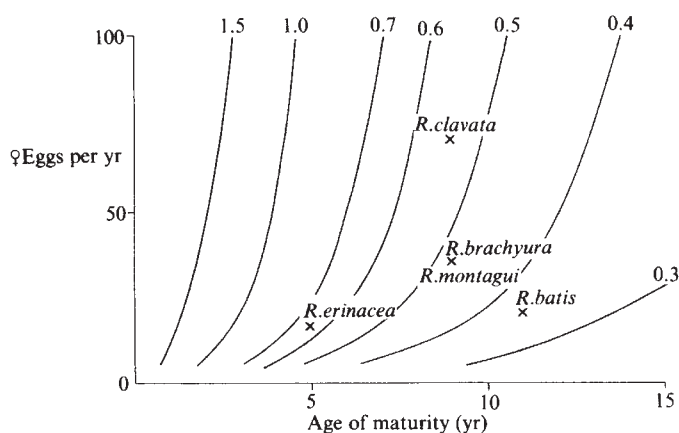


Fig. 2 Value of total mortality ($Z_i = Z_m$) beyond which the population will collapse, for different values of fecundity and age of maturity.

to 1.2 (total instantaneous rates on an annual basis⁶). The disappearance of *R. batis* is therefore not surprising, but that this took place virtually unnoticed or without comment in the fisheries literature, perhaps is surprising. Other ray species have also declined⁶, but are less vulnerable, as can be seen from Fig. 2. This shows the highest total mortality which the different species could withstand without collapsing, as a function of fecundity and age of maturity. It is assumed that $Z_m = Z_i$, but young *R. clavata*, *R. brachyura* and *R. montagui* may be less vulnerable to fishing mortality than *R. batis*, as they are smaller.

The prediction from Fig. 2 that *R. erinacea* would withstand the highest mortality of any of these species, in spite of having the lowest fecundity, emphasizes that net survival to maturity rather than fecundity *per se* governs the response to exploitation. For *R. batis* the only effective protection is probably a complete halt to all kinds of fishery in which it may be caught. Because this course of action is unrealistic, it has to be accepted that this species and others like it will be fished out as a consequence of the exploitation of other demersal fish.

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Infant-related influences on birth intervals in rhesus monkeys

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That a mother's relationship with her infant could influence her subsequent reproductive history can be argued as follows. A rhesus monkey infant whose next sibling is to be born in a succeeding birth season¹ could compete with it by trying to postpone the date when its mother next conceives. If the mother preferred a shorter delay than the infant², processes of conflict³ and negotiation⁴ involving the two could show in some of the behavioural interactions constituting their relationship⁵. For example, a mother could try to hasten the onset of her next pregnancy by trying to promote more independent behaviour in her infant, perhaps by rejecting some of its bids for contact^{3,4}. We report here studies on our rhesus monkey colony and other colonies which support this argument.

We compared two reproductive groups of mothers in our colony¹: 'quick' mothers, who have an infant in the present birth season¹ and will conceive another in the coming mating season, with 'slow' mothers, who bear an infant this season but will not conceive another in the coming mating season. The two groups differed as follows: current infants of quick mothers included a higher proportion of males than did current infants of slow mothers (Fig. 1, $P < 0.01$ by Median test⁶, excluding two sons and four daughters whose mothers have not yet produced another full-term infant); current infants of quick mothers were more active, crossing grid lines in their cages more often⁷ during their first 12 weeks ($P = 0.048$, 0.008 and 0.035 in weeks 2, 4 and 8 respectively, Mann Whitney U-test); and quick mothers rejected^{7,8} their infants more often in seven of the eight observation weeks up to week 20 (Fig. 2, $P < 0.10$ in weeks 2-10, $P < 0.018$ in week 6 and < 0.011 in week 10).

In Bernstein's captive colony of rhesus monkeys⁹, more mothers of sons than of daughters were 'quick' (personal communication). Colvin (personal communication) found the same in the free-ranging rhesus population on Cayo Santiago in 1978-79 ($P = 0.0072$, Fisher exact probability test), but it has not been found in free-ranging populations studied over longer periods, either on Cayo Santiago (ref. 10, and Sade and Berman, personal communication) or on La Parguera (Leighton-Shapiro, personal communication). At this stage we presume that the special conditions in captive colonies of rhesus monkeys, and in

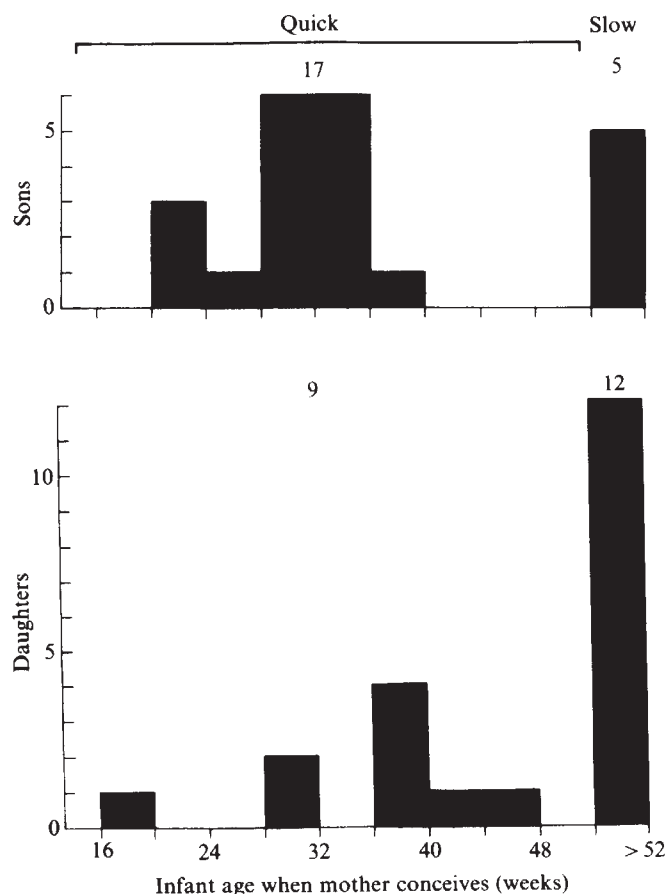


Fig. 1 Ages in weeks of current sons and daughters when their mothers conceive infants to be born in the next birth season (quick), and numbers of sons and daughters whose mothers fail to give birth in the next season (slow). The conception date of each infant to be born next season was calculated by subtracting 168 days²⁶ from its birthdate. The 43 infants and their mothers were healthy through at least the year following the births of the current infants, and those births occurring in the next season were full term¹. Our rhesus monkey colony has six separately caged groups, each with one adult male, 2-5 adult females, and immature individuals which have been born into the group¹.

some years in free-ranging populations, determine that an infant's sex becomes associated with its mother's next conception date.

In our colony, the tendency of mothers of males to conceive sooner could be explained in terms of infant activity level, if male infants as a group are more active. We found this to be the case in the infants' second week when more sons than daughters entered at least one new cage section during 6 h of observation ($P = 0.023$, Fisher exact probability test). Thereafter entry rates into new cage sections no longer differentiated male and female infants, but in all observation weeks during their first half year, males initiated more of the play episodes in which they were involved (see also ref. 11). (After week 2, when all infants can move freely, we do not expect males to enter more cage sections than females, because male social play often includes much rough-and-tumble activity in one place, whereas female play may include more chasing to and fro¹¹.)

If infant activity is associated with subsequent maternal conception date, this association should also show within the infant sex classes separately. Taking sons alone, those with quick mothers were more active in weeks 2, 4 and 6. Daughters with quick mothers were more active in all 10 observation weeks up to week 28 ($P < 0.022$ in weeks 8, 10, 16 and 20 by Mann Whitney U-test). Also, in all these weeks, daughters of quick mothers spent less time on their mothers' nipples ($P < 0.05$ in week 20, Mann Whitney U-test). If an infant's sucking behaviour is related to other aspects of its activity, one mechanism promoting early conception could involve sucking¹²⁻¹⁴ (at least in our mother-daughter dyads).

The association between maternal rejection scores^{7,8,15} and subsequent conception held true for sons and daughters separately throughout their first 10 weeks (Fig. 2), quick mothers being more rejecting. In a group of savannah baboons, less restrictive mothers had a shorter postpartum amenorrhea, although they did not conceive again sooner¹⁶. Baboon mothers who were less restrictive were also more rejecting^{4,17}. Associations between a mother's social position in her group and her fertility are also to be expected, and occur for our mothers of males (work in progress).

We provisionally suggest the following mechanism to explain why mothers who reject their infants should be more likely to conceive relatively early. A mother both responds to and tests the progress of her infant, conceiving earlier, the better its progress seems to be². That mothers could be affected by their infants' activity is suggested by the correlation between infant activity and maternal rejection rate in the infants' early weeks (Spearman rank-order correlation coefficient = 0.65, 0.80, 0.49 and 0.43 in weeks 2, 4, 6 and 8 respectively, $P < 0.007$). Mothers could even test their infants' capacity for activity by persisting in their rejecting behaviour until the infants begin to protest. The view of progress so acquired by the mother should be quite reliable, for by falsifying it² the infant could act to its own disadvantage. A clinging and sluggish infant would miss opportunities for play and exploration¹⁸.

This hypothesis does not explain why more mothers with male infants should subsequently be quick to conceive, unless we accept the greater activity of the sons at 2 weeks as a sufficient explanation. However, one or more of the following infant sex differences, expressed *in utero* and later, could also be effective. Thus our rhesus mothers pregnant with male fetuses received fewer threats, chases and attacks than those pregnant with females (work in progress), and pigtail macaque mothers (*Macaca nemestrina*) carrying male fetuses were less likely to require medical treatment (usually for bite wounds from social companions) than those carrying females¹⁹. Chamove's stump-tail macaques (*M. arctoides*), whose infants were removed at birth, bore subsequent infants sooner if they were male (personal communication). Rhesus mothers caged singly with their infants²⁰, and in our social groups (work in progress) played more often with male than with female infants, and male infants initiated a greater proportion of their play sessions than females¹¹.

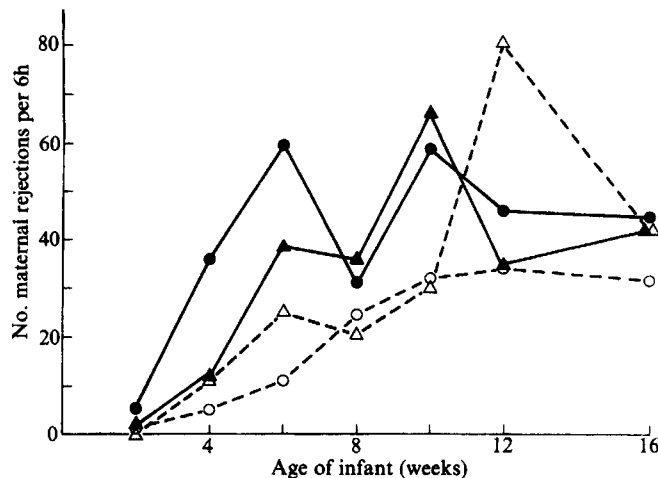


Fig. 2 Maternal rejection of daughters and sons through the infants' first 16 weeks, depending on whether mothers will conceive again between the infants' 16th and 52nd weeks (quick: ●, daughters; ▲, sons), or later (slow: ○, daughters; △, sons). Each data point is a median frequency of rejection from 6 h of observation¹⁵ of one of the groups. A rejection^{7,8,15} was scored each time a mother hit, pushed or bit her infant, blocked its access to her ventrum and/or nipple, or broke contact with it within 5 s of the infant initiating a period of physical contact with her. A rejection was also scored when the infant was already in contact with the mother's body and she blocked its access to her ventrum and/or nipple. The 43 infants were born to 20 mothers over a 7-yr period, and were treated as if they were statistically independent⁶. This is justifiable because we found that none of our six social groups, nor any of our families within social groups consistently produced infants that were male rather than female, or quick rather than slow.

The idea that mothers in good condition should produce sons rather than daughters²¹ suggests another explanation of our results. If old mothers were in poor condition and therefore 'slow', maternal condition could explain our observation that mothers of daughters tended to be slow. But many of our old mothers were vigorously maintaining high dominance status in their social groups, and were as quick or slow according to the sex of their infants as the remainder of our mothers.

High maternal rank could be the common factor underlying a tendency to have daughters⁴ and then to be slow. However, our high-ranking mothers of sons were among our 'quickest' mothers of all (work in progress).

The survival value to mother, and infant, of the mother being able to respond to and test her infant's progress is easily seen. It is less obvious, in terms of evolutionary theories, about how parents should invest care in their current and future offspring^{2,21-24}, why mothers of male rhesus infants, which are the slightly heavier sex at birth, should conceive again sooner. (Males are heavier in all studies known to us, but only statistically significant in that of Goy, personal communication.) In red deer (*Cervus elaphus*) the heavier stag calves are believed to be more costly to rear and in accordance with this idea their mothers are more likely to have a subsequent barren year²⁵. We wish to note the contrast provided by our rhesus monkeys and make a plea for comparable studies of more species.

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Hepatitis B polypeptide vaccine preparation in micelle form

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The immunoprophylaxis of hepatitis B is hampered by the lack of a technique for growing hepatitis B virus (HBV) in tissue culture. Plasma from persistently infected individuals¹, one source of viral antigen, contains characteristic 22-nm spherical particles which share a common antigen (the hepatitis B surface antigen or HBsAg) with the outer envelope of the 42-nm double-shelled DNA virus. Highly purified inactivated 22-nm particles have been shown to be safe and to confer protective immunity against HBV in a recent large-scale clinical trial². We have already described the extraction from the particles of a complex of two proteins which are antigenic determinants of HBV—the polypeptide with molecular weight (MW) between 22,000 and 24,000 (called p23) and the glycosylated polypeptide (called gp28) with MW in the range 26,000–29,000 which is thought to be the glycosylated form of p23. We now report the preparation from this complex of water-soluble protein micelles which may be a suitable basis for a second-generation hepatitis B vaccine.

In the preparation of viral vaccines, highly purified viral antigens may be significantly safer than other preparations. Many viral vaccines contain not only specific viral antigens but also components derived from the host tissues in which virus has been grown and against which those to whom the vaccine is administered may react. The availability of a chemically defined polypeptide viral antigen may be especially important for HBV, for there are several reports of the persistent presence in preparations of 22-nm spherical particles of host-serum proteins^{3,4} which may inhibit the immune response or even induce immunological side reactions⁵. Sources of HBV antigen such as the hepatoma cell line PLC/PRF/5 (ref. 6) and *Escherichia coli* containing cloned viral DNA (ref. 7) are similarly contaminated. The advantages of vaccines based on pure antigens have, however, been demonstrated with influenza, where a vaccine consisting of the neuraminidase and haemagglutinin subunits has been shown to be adequately immunogenic when given in two doses and less stimulating of side reactions than the whole virus vaccine.

In our search for a chemically characterized antigen of HBV, we have concentrated on the surface antigen (HBsAg) present in

the 22-nm particles in plasma, which consist of lipid and several viral and host proteins (including, for example, serum albumin^{8,9}). The two peptides p23 and gp28 (refs 8, 10), which form a 49,000-MW complex in non-reducing conditions¹¹, have identical amino acid sequences at the N-terminal end¹² and recent nucleotide sequences¹³ suggest that there is an internal sequence of 19 hydrophobic amino acids which may be the site of the intercalation of the proteins in the 22-nm particles. It seems reasonable, therefore, to consider gp28 and p23 as integral membrane proteins, by definition partially hydrophobic and insoluble in aqueous media in the absence of detergents¹⁴. Complete solubilization of the integral proteins of viral membranes, with minimal loss of serological activity, has been most successfully achieved by the use of non-ionic detergents such as Triton X-100^{15,16}. However, the polypeptides are poor immunogens in a monomeric form in detergent solutions¹⁷. Recently, a method of detergent removal has been described which allows the amphiphilic proteins to reassociate through their hydrophobic portions into a protein micelle structure, which remains water soluble due to the presence on its outer surface of the hydrophilic portions of the molecules¹⁸.

Micelles prepared from Semliki Forest virus membrane proteins were highly effective in inducing a protective response to the virus in mice¹⁷. Applying this type of strategy to 22-nm HBsAg spheres purified from the serum of a carrier chimpanzee, we have previously reported the extraction of a complex of gp28 and p23 by Triton X-100 solubilization and affinity chromatography⁸. The 3.9S complex of p23 and gp28 was prepared essentially according to this method⁸. Purified 22-nm spheres, labelled with ¹²⁵I, were disrupted with 2% Triton X-100. The complex was separated from other particle constituents by

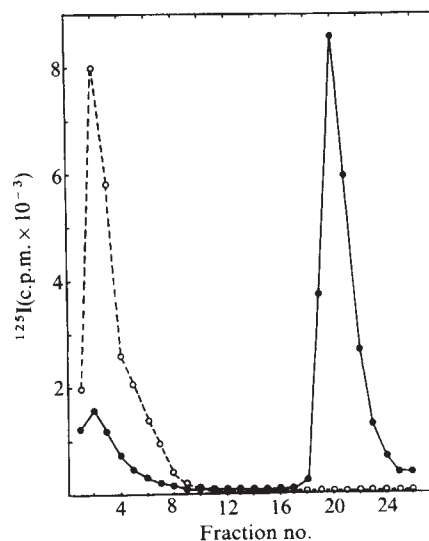


Fig. 1 Sedimentation of solubilized p23 and gp28 in 20–50% linear sucrose gradients in the absence (●) or presence (○) of 2% Triton X-100 throughout the gradient. The 22-nm HBsAg particles were purified from the serum of a persistently infected chimpanzee and radiolabelled with the Bolton and Hunter reagent, as previously described²². ¹²⁵I-HBsAg in 0.01 M Tris buffer was made 2% with Triton X-100 and 0.5 M NaCl, and incubated overnight at 37 °C (ref. 8). The solubilized material was passed through a column of Con A-Sepharose equilibrated in the complete reaction buffer supplemented with 1 mM CaCl₂ and MnCl₂. The gp28–p23 complex was eluted with α-methylmannoside and layered directly onto 20–50% linear gradients of sucrose in TNE buffer (0.50 M Tris-HCl, pH 7.4, 0.14 M NaCl, 0.001 M EDTA). After centrifugation at 220,000g for 24 h at 15 °C in a Beckman SW40 rotor, 0.5-ml fractions were collected from the top of the tube and counted to locate the position of the polypeptides. The direction of sedimentation is from left to right.

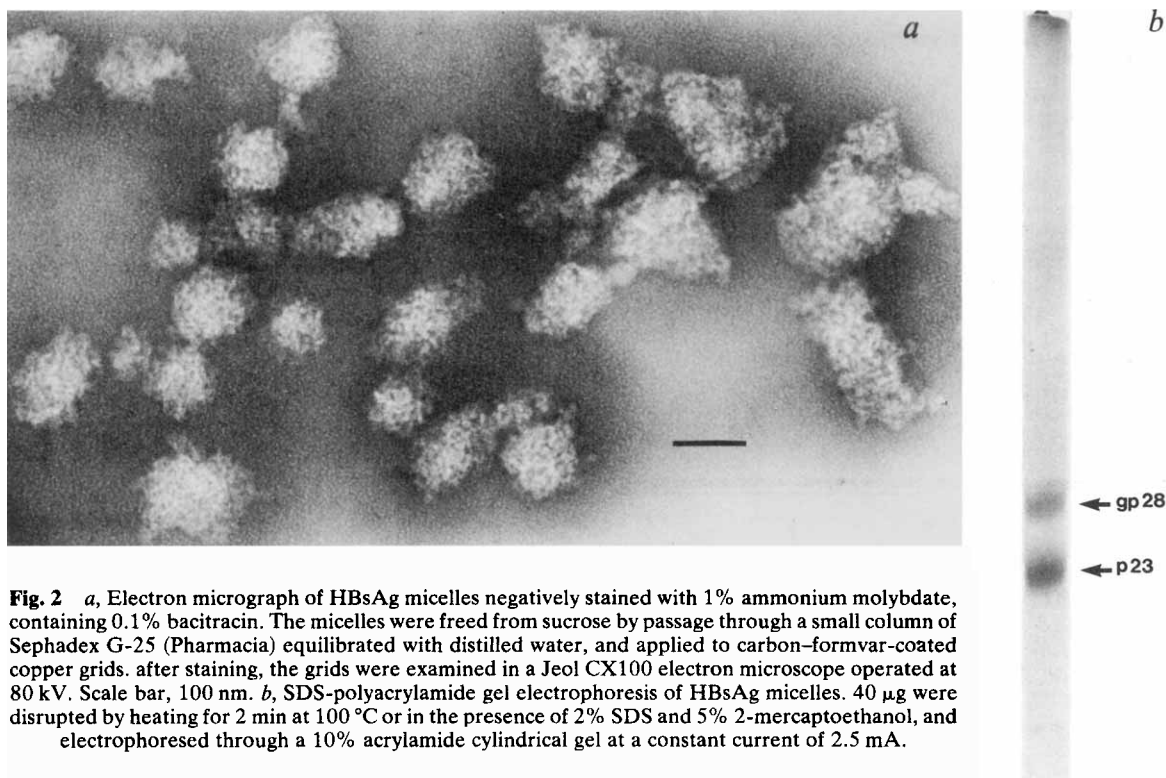


Fig. 2 *a*, Electron micrograph of HBsAg micelles negatively stained with 1% ammonium molybdate, containing 0.1% bacitracin. The micelles were freed from sucrose by passage through a small column of Sephadex G-25 (Pharmacia) equilibrated with distilled water, and applied to carbon-formvar-coated copper grids. After staining, the grids were examined in a Jeol CX100 electron microscope operated at 80 kV. Scale bar, 100 nm. *b*, SDS-polyacrylamide gel electrophoresis of HBsAg micelles. 40 μ g were disrupted by heating for 2 min at 100 °C or in the presence of 2% SDS and 5% 2-mercaptoethanol, and electrophoresed through a 10% acrylamide cylindrical gel at a constant current of 2.5 mA.

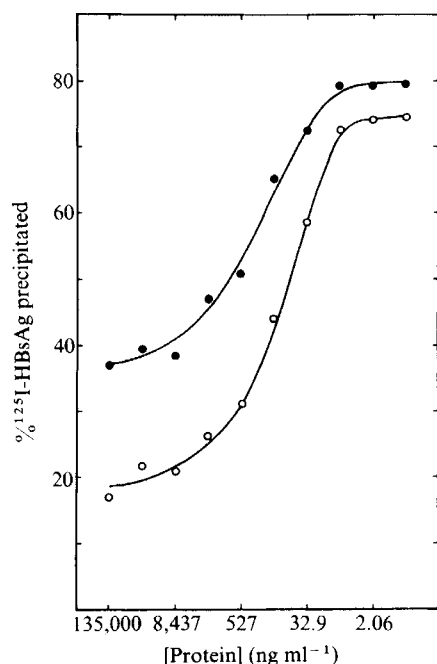


Fig. 3 Competitive radioimmunoassay of HBsAg micelles (●) and intact 22-nm particles (○). Both samples were adjusted to 360 μ g ml⁻¹ of protein and serially diluted fourfold in phosphate-buffered saline (PBS) containing 0.5% of bovine serum albumin (BSA). A 150- μ l aliquot of each dilution was mixed with an equal volume of a 1:100 dilution of rabbit hepatitis B surface antibody (anti-HBs) (Hoechst) and incubated at 37 °C for 3 h. ¹²⁵I-HBsAg (intact 22-nm particles, 10 μ l, 24,000 c.p.m.) was then added and incubation was continued overnight at room temperature. A 150- μ l aliquot of protein A-Sepharose (Pharmacia) at a concentration of 5% in PBS-BSA was then added and the samples were shaken vigorously for 1 h at room temperature. The Sepharose was pelleted by low-speed centrifugation and the supernatants and pellets separated and counted. The results are expressed as the percentage of total counts associated with the precipitated immune complexes.

affinity chromatography on concanavalin A (Con A)-Sepharose in the presence of the detergent, and retained specific reactivity with anti-HBs after elution with α -methyl mannoside. The complex in detergent solution was then centrifuged into two 20–50% w/v sucrose gradients, one of which contained 2% Triton X-100 throughout (Fig. 1). In the gradient containing detergent, all the radioactivity remained at the top; in the absence of detergent a peak of ¹²⁵I-labelled protein was found two-thirds of the way down the gradient¹⁸. These results indicated that sedimentation of the complex into a detergent-free gradient separated the polypeptides from the detergent and allowed them to reassociate during centrifugation into a particulate protein micelle form with an increased sedimentation coefficient.

The buoyant density of the micelles in caesium chloride was 1.25 g ml⁻¹ compared with a density of 1.19 g ml⁻¹ for intact 22-nm particles, an increase consistent with the removal of most of the lipid by the solubilization procedure. Electron microscopy showed that the micelles were pleomorphic and fluffy in appearance, with diameters in the range of 60–200 nm (mean 120 nm) (Fig. 2*a*). Immune electron microscopy showed that the micelles could be both self-aggregated and co-aggregated with intact 22-nm particles by anti-HBs¹⁹. Polyacrylamide gel electrophoresis (Fig. 2*b*) showed that both gp28 and p23 were present in the same proportions as in the original detergent extract, indicating that the micelles were not formed preferentially from one or other polypeptide. Although the possibility that each polypeptide might have segregated into different micelles was not examined, it seems unlikely in view of the recent demonstration of a disulphide linkage between them¹¹. Gp28 and p23 together constituted 40% of the total protein of the purified 22-nm particles used as starting material in these experiments (see ref. 8). Ninety per cent of the gp28–p23 complex was recovered from the Con A-Sepharose column, and the final yield of the two polypeptides in micelle form was estimated to be 60–70% of the amount originally present in the intact 22-nm particles. The good recovery of total protein confirms the suitability of the method for large-scale production of a potential vaccine.

The degree of antigenic reactivity and specificity of the micelles was assessed by subjecting them to competition with

intact HBsAg particles for anti-HBs in a radioimmuno-precipitation test (Fig. 3). The micelles competed effectively with intact 22-nm forms, although the dilution curve had a slightly shallower slope than that obtained in a control experiment using unlabelled intact 22-nm particles as the competing antigen. Such a difference, indicative of altered protein conformation, was to be expected following removal of lipid and reassociation of the polypeptides into a purely protein form. It is difficult to give a precise estimate for the recovery of serological activity due to the difference in slope, but the competitive ability of the micelles was about 40% that of the intact 22-nm particle per weight of protein. The differences in surface area, and in surface distribution of antigenic sites between the two types of particles, may account for this discrepancy.

The immunogenicity of the micelles was compared with that of intact 22-nm particles in a mouse potency test, as it has been suggested that the serological response in mice may be a useful indicator of the immunogenic potential of candidate hepatitis B vaccines²⁰. Groups of six SWR/J mice were inoculated with three serial doses of 5, 10 or 20 µg of each preparation in alum-adsorbed form, and the antibody levels in their sera measured by a protein A radioimmunoassay. The results obtained are shown in Fig. 4 in two forms. Figure 4a is a plot of the mean values of the serial dilutions of all sera obtained from mice inoculated with 10-µg doses of micelles or intact particles.

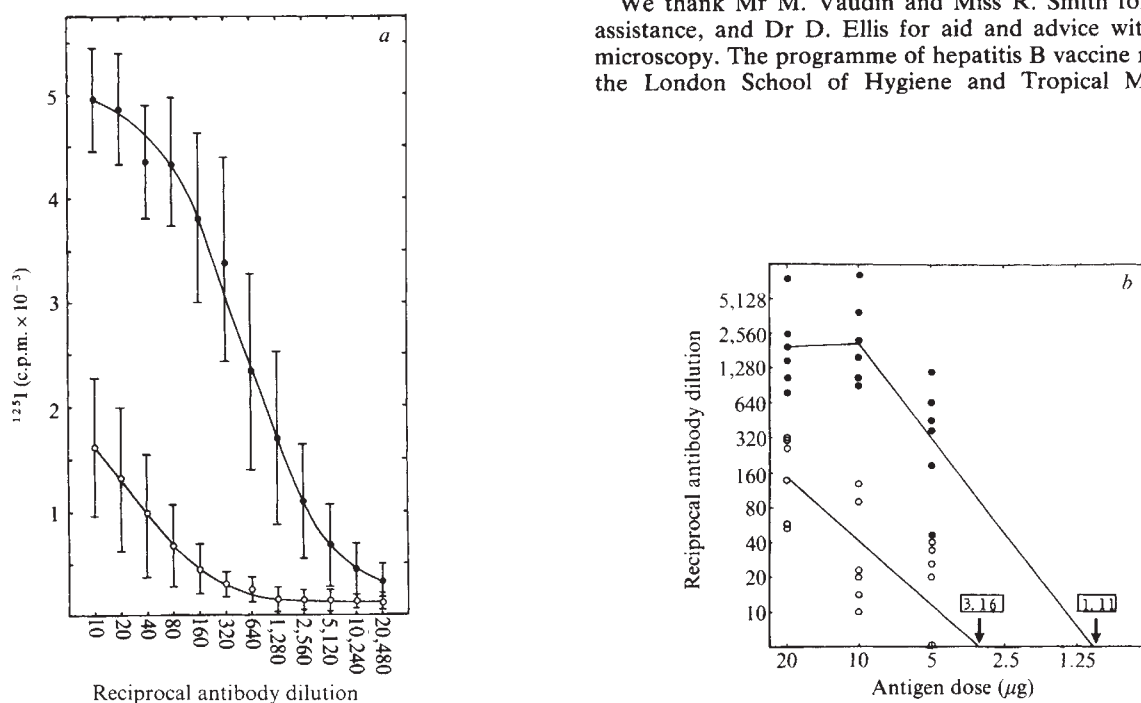


Fig. 4 Potency testing of HBsAg micelles and 22-nm particles. Both types of preparation were dialysed into 0.01 M phosphate buffer, pH 6.6, and adjusted to a final concentration of 200 µg of protein per ml. A 1-ml aliquot of a 2% suspension of aluminium phosphate was added to 6 ml of each sample and the protein adsorbed to the gel by mixing the suspensions for 3 h. The alum was then centrifuged and washed once with PBS. Over 95% of the antigen was adsorbed by the alum. The alum was resuspended in PBS to a final concentration of 0.4%. The suspensions were then diluted appropriately with 0.4% alum in PBS so that both the volume and alum content of the inocula were the same for all antigen doses. Six mice per dose were injected intraperitoneally with 20, 10 or 5 µg of either the intact 22-nm particle or the micelles in 100-µl volumes. Two booster doses were given at weekly intervals. The mice were bled 11 days after the last inoculation and the titres of anti-HBs determined using a protein A radioimmunoassay. Flexible microtitre plates (Dynatech) were coated with 100 µl per well of HBsAg at a concentration of 10 µg ml⁻¹ in carbonate buffer, pH 9.5, by overnight incubation at 4 °C. The antigen was removed and 100 µl per well of fetal calf serum added to block any remaining adsorption sites. After 2 h at 4 °C, the fetal calf serum was removed and the plates washed with PBS. 100-µl aliquots of serial twofold dilutions of the test serum in PBS-BSA were incubated in the plate for 2 h at 37 °C, the wells were drained and rinsed and 100 µl of ¹²⁵I-protein A was added to each. After 1 h at 37 °C, the plate was drained, washed several times with PBS and divided into individual wells for counting in a γ spectrometer. Protein A (Pharmacia) was iodinated by the chloramine-T method to a specific activity of 20–40 µCi µg⁻¹. *a*, Protein A radioimmunoassay of anti-HBs in the sera of mice inoculated with 10-µg doses of either micelles (●) or intact 22-nm particles (○). The sera were initially diluted 1:10 in PBS–0.5% BSA. The points represent the mean of six sera, and the vertical lines represent the standard deviations. *b*, Geometric mean antibody titres at different dose levels of HBsAg micelles (●) or 22-nm particles (○). Titres are expressed as the reciprocal of the dilution of antibody required to bind a given amount of radioactive protein A. Each point represents the titre in a single animal. The antigen extinction doses in each case are indicated.

Consistently higher levels of anti-HBs were induced in animals receiving micelles, possibly also with a higher affinity, as indicated by the steeper slope of the dilution curve compared with that of the sera from mice receiving intact particles, although direct affinity measurements were not made. This difference was seen at all dose levels tested. Figure 4b shows the antibody titres against dose for all animals, each point representing the titre of an individual serum. Extrapolation of the lines representing geometric mean titres of antibody gave antigen extinction values of 1.11 µg and 3.16 µg for HBsAg micelles and intact particles, respectively. Several factors may, singly or in combination, account for the greater immunogenicity of the micelles, including their large size, altered distribution of antigenic sites, and the absence in them of host-derived serum proteins such as albumin. The results presented here show that the antigenic complex of gp28 and p23 can be readily isolated in high yield from intact HBsAg 22-nm particles, and that this complex can be reassociated into a micellar form as has been described for other viral envelope proteins^{18,21}. The chemical purity, specific serological activity and powerful immunogenicity of the micelles, taken together with the ease of their preparation on a large scale, strongly favour their development as an alternative second-generation hepatitis B vaccine. This physical form may indeed also be suitable for vaccines prepared using HBsAg polypeptides produced by the expression of cloned hepatitis B virus DNA in *E. coli* or other hosts⁷. Experiments to test the immunogenicity, safety and protective capacity of HBsAg micelles in primates are in progress.

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Ethyl β -carboline carboxylate lowers seizure threshold and antagonizes flurazepam-induced sedation in rats

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The mammalian central nervous system possesses specific high-affinity binding sites for the benzodiazepines and considerable evidence suggests that these binding sites are the pharmacological receptors through which these compounds act^{1–6}. Recently⁷, ethyl β -carboline-3-carboxylate (β -CCE) has been identified in both human urine and rat brain. β -CCE may be closely related to the endogenous ligand for the benzodiazepine receptor—it shows an affinity for the receptor of the same order as that of clonazepam, one of the most potent benzodiazepines, and is the first non-diazepinoid structure to be identified with an affinity in the nanomolar range. Furthermore, it is selective for the benzodiazepine receptor⁷. Clinically and in animal studies, benzodiazepines have anti-convulsant, hypnotic and anxiolytic actions⁸. We have therefore investigated whether β -CCE exhibits any of these properties in rats. We report here that, in contrast to the benzodiazepines, β -CCE lowers seizure threshold and reverses the sedative effect of flurazepam. If β -CCE has a close structural relationship to the endogenous ligand, benzodiazepines may be antagonistic at the receptor site.

β -CCE was synthesized by condensation of L-tryptophan with glyoxylic acid, esterification of the product with ethanol-HCl and oxidation with chloranil. The purity of the final product was >95%.

The effect of β -CCE on seizure threshold in rats was determined by measuring the dose of bicuculline, infused through a tail vein, required to elicit a seizure⁹. The β -CCE was dis-

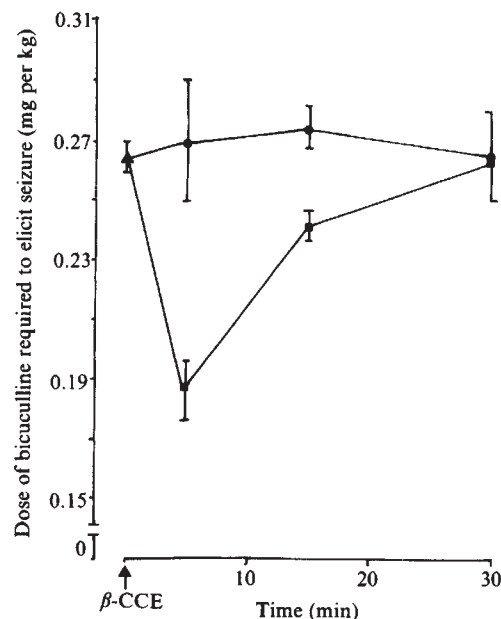


Fig. 1 Effect of β -CCE on the seizure threshold to bicuculline at various times following administration. Results show the seizure threshold for a group of uninjected rats ($\blacktriangle$) and for groups of vehicle-injected ($\bullet$) and β -CCE (5 mg per kg)-injected ($\blacksquare$) animals. Results show mean $\pm$ s.e.m. for groups of four or more rats. The β -CCE group was significantly different from the vehicle-injected group— $P < 0.001$ at 5 min and $P < 0.005$ at 15 min.

solved in ethanol, diluted with propylene glycol (ethanol/propylene glycol, 1:2) and injected intravenously (i.v.). Control rats received the vehicle alone. Each rat was only used once for any determination.

When β -CCE was given i.v. at a dose of 5 mg per kg, there was a significant (31%) decrease in the dose of bicuculline required to elicit a seizure 5 min later. Only a small lowering of threshold was seen 15 min after administration and no effect was observed after 30 min (Fig. 1).

Pretreatment of rats with a dose of β -CCE as low as 0.5 mg per kg i.v. had a statistically significant effect in lowering seizure threshold 5 min later and the drug displayed a conventional log dose-response curve (Fig. 2). The effect of β -CCE on lowering seizure threshold plateaued at about 2.5 mg per kg, suggesting that the compound itself will not induce convulsions even at high doses.

When β -CCE (5 mg per kg) was given intraperitoneally, no effect on the bicuculline seizure threshold was seen 5 min later (vehicle 0.28 ± 0.02 mg per kg, $n = 4$; β -CCE 0.32 ± 0.04 mg per kg, $n = 4$). This could be due to rapid inactivation and may explain the short duration of action of the drug even when given i.v.

Benzodiazepine receptors are thought to be functionally linked to γ -aminobutyric acid (GABA) receptors^{10,11} and bicuculline is a GABA receptor antagonist¹². We therefore examined the effect of β -CCE on the seizure threshold using pentylenetetrazol (PTZ), as this convulsant agent also has GABA-antagonist properties¹³. We also investigated the effect on β -CCE on the seizure response to strychnine, a glycine antagonist, to see whether the action of β -CCE was specific to drugs acting on GABA functions. Five minutes after administration of β -CCE at a dose of either 5 or 1 mg per kg the seizure threshold to PTZ was lowered by a value (30%) comparable with that seen using bicuculline (vehicle 23 ± 3 (5); β -CCE (5 mg per kg) 16 ± 3 (6); β -CCE (1 mg per kg) 15 ± 3 (4), expressed as dose of PTZ (mg per kg) to elicit a seizure; β -CCE different from vehicle, $P < 0.001$, at both doses).

In contrast, no change was seen in the strychnine seizure threshold (vehicle 2.7 ± 0.2 (4); β -CCE (5 mg per kg) 2.7 ± 0.1 (4), expressed as dose of strychnine (mg per kg) required to elicit seizure). Following flurazepam (10 mg per kg) the PTZ seizure threshold is raised whereas the strychnine threshold is unaltered¹⁴.

When rats were given flurazepam (20 mg per kg intraperitoneally) they rapidly showed sedation and, in contrast to control animals, displayed very little 'exploratory' locomotor activity when placed in a new cage. This was reflected in low counts on Automex activity meters (Fig. 3). Injection of vehicle had no significant effect on this low level of activity. In contrast,

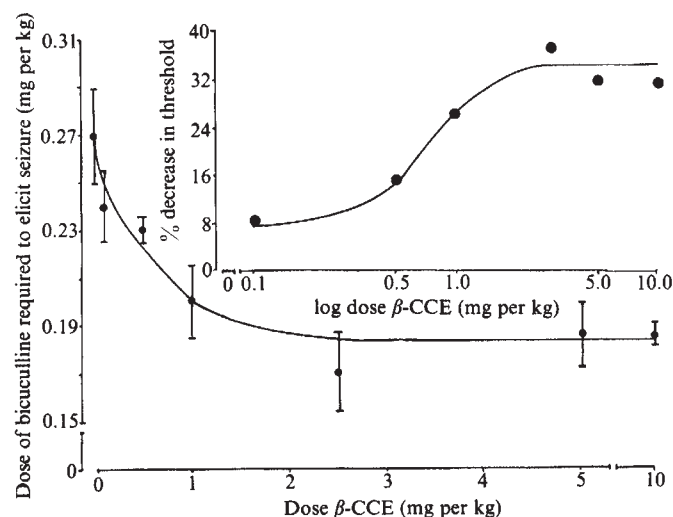


Fig. 2 Effect of dose of β -CCE on bicuculline-induced seizure threshold. Main graph shows dose of bicuculline required to elicit seizure 5 min after injection of various doses of β -CCE. All doses of drug were given in the same volume of vehicle (1 ml per kg). Zero value shows threshold following vehicle alone, which is not different from a saline-injected group (0.26 ± 0.01 , $n = 4$). Different from vehicle control: 10, 5 and 2.5 mg per kg, $P < 0.001$; 1 mg per kg, $P < 0.01$; 0.5 mg per kg, $P < 0.02$; not significantly different: 0.1 mg per kg; four or more observations at each point. Inset shows the log dose-response curve of β -CCE measured against the mean percentage decrease in seizure threshold.

β -CCE (5 mg per kg i.v.) administration partially reversed the sedative effect of the flurazepam for about 10 min after injection (Fig. 3). β -CCE (5 mg per kg i.v.) alone did not produce an increase in locomotor activity compared with rats given vehicle. This suggests, therefore, that the increase in activity in flurazepam-treated rats receiving β -CCE resulted from an antagonism of the sedative effect of flurazepam.

There have been extensive investigations of the biochemical and behavioural effects of both natural and synthetic β -carboline, some of which have been shown to have convulsant or anticonvulsant properties^{15,16}. However, of the several β -carboline derivatives studied by Braestrup *et al.*⁷, only β -CCE and methyl β -carboline-3-carboxylate showed high-affinity binding (comparable with diazepam) to the benzodiazepine receptor. Although, as these authors admit, it is unlikely that β -CCE is the endogenous ligand, the ethyl ester group having been added during the isolation and purification process, they nevertheless suggest that it may be closely related to the endogenous ligand^{7,17}.

Sieghart and Karobath¹⁸ have recently demonstrated a heterogeneity of benzodiazepine receptors separable, after photoaffinity labelling, by SDS-polyacrylamide gel electrophoresis into two major bands with apparent molecular weights

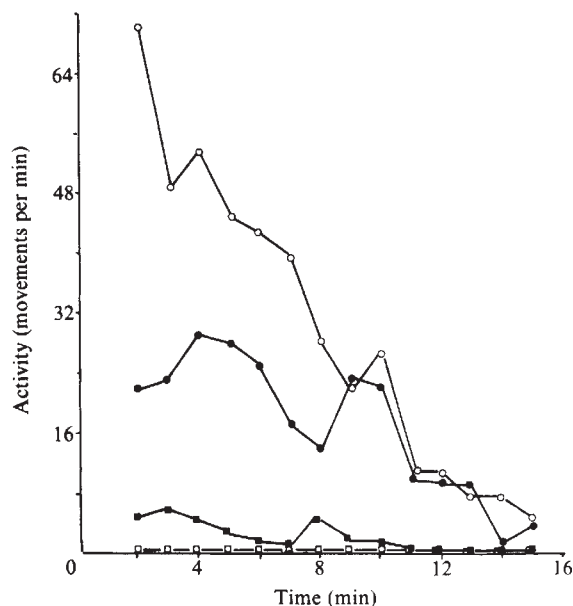


Fig. 3 The activity response of rats placed in cages on Automex activity meters following pretreatment with flurazepam (20 mg per kg, $n = 4$, $\square$), flurazepam plus vehicle ($n = 6$, $\blacksquare$), flurazepam plus β -CCE (5 mg per kg i.v., $n = 7$, $\bullet$) or saline ($n = 4$, $\circ$). Saline or flurazepam were given 15 min before the β -CCE or vehicle. Results show activity measured in recorded counts per min against time (min) after β -CCE or vehicle. Results at 2 min show response in the period 1–2 min post-injection. Flurazepam plus β -CCE is significantly different from flurazepam plus vehicle group at $P < 0.05$ or better for the points 2–10 min inclusive. Flurazepam plus vehicle was at no time significantly different from flurazepam alone.

of 51,000 (P_{51}) and 55,000 (P_{55}). The receptor subpopulations show a differential regional distribution, the cerebellum containing mainly P_{51} and the hippocampus approximately equal amounts of P_{51} and P_{55} . Data¹⁹ showing a lower IC_{50} for β -CCE in the cerebellum than hippocampus, indicates a greater affinity of β -CCE for the P_{51} than the P_{55} -subtype, but this difference in affinity is probably less than one order of magnitude. Such a small difference prevents a detailed interpretation of experiments, such as those reported here, performed on two different behavioural paradigms. We can conclude that the β -CCE has effects opposed to the benzodiazepines, but not that the reversal of the flurazepam-induced sedation and the convulsant effects of β -CCE are mediated through the same subpopulation of benzodiazepine receptors.

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Peripheral nerve grafts in hereditary leukodystrophic mutant mice (twitcher)

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The twitcher mouse is a mutant affected by a form of leukodystrophy which shows close similarities to human globoid cell (Krabbe's) leukodystrophy. Transmission is by an autosomal recessive gene *twi*. Progressive loss of myelin sheaths from both central and peripheral nervous systems and the presence of inclusion-laden macrophages are characteristic findings. Morphological features of the twitcher have been described by Duchon *et al.*¹. Nerve iso- and allografting have been used to determine the roles of axon and Schwann cell in a number of mouse² and human³ nerve abnormalities. Schwann cells in a graft proliferate and become associated with regenerating host axons which grow through the graft into the host distal stump. In the twitcher, peripheral nerve axons do not degenerate but are thinner than normal, although there is considerable axonal degeneration in the central nervous system. In 15-day-old mutants, inclusions have been found in Schwann cells associated with apparently normal myelin sheaths. Grafting experiments might show whether the phenotype of this mutant is fully expressed in the Schwann cell, or if axons are also involved. In previous experiments, survival of transplanted Schwann cells was achieved by the use of T cell-suppressed^{2,3} or nude mice⁴. We report here that a twitcher nerve transplanted in immunologically un-suppressed animals reproduces all the characteristic features of leukodystrophy and conversely that Schwann cells from unaffected mice can produce normal myelin when associated with twitcher axons.

Myelin seems to form normally until the mice are ~15 days old although the myelin sheaths and axons are rather thinner than those of normal littermates. Later, the myelin sheaths start to degenerate and macrophages containing inclusions appear. By 3–4 weeks, peripheral nerves are macroscopically swollen; many axons are demyelinating or bare of myelin and others have thin myelin sheaths indicative of remyelination; the fibres are widely separated by endoneurial fluid and by large numbers of macrophages. There are various inclusions in macrophages and Schwann cells—those of particular interest are the multi-angular bodies and twisted tubules (Fig. 1*a, b*) also found in the human⁵ and canine⁶ forms of globoid leukodystrophy. Deficiency of the enzyme galactosylcerebrosidase was found in human cases of Krabbe's disease^{7,8} and also more recently in the twitcher mouse⁹, establishing the validity of the mutant as a model for the human disease.

Four twitcher litters (aged 40–45 days) were used, including normal and affected mice, and sciatic nerve grafts were carried out between littermates to limit as much as possible any immunological differences. Normal littermates which received twitcher transplants, originally used as controls, were expected to reject their grafts but in most cases they showed no signs of rejection. Grafts were also made from normal mice into twitchers but the life expectancy of the twitcher is so short that transplantation was carried out on 7-day-old littermates. At this age mutants were indistinguishable from their normal littermates and grafts were done at random. Successful grafts of normal sciatic nerves into twitcher sciatic nerves were achieved in two cases which survived for 35 and 37 days.

A total of 25 animals (20 normal and 5 twitchers) were grafted successfully. The results of twitcher sciatic nerve transplants into normal recipients 1–6 months after grafting are reported as well as those of the reverse experiment with 35- and 37-days

survival. To exclude the possibility of migration of Schwann cells from the proximal and distal stump into the transplant, two normal mice had the sciatic nerve severed at mid-thigh. After 48 h, at the peak of Schwann cell mitosis¹⁰, ³H-thymidine (4 µCi per g body weight) was injected intraperitoneally in each animal. After a further 24 h the two mice received a transplant from a twitcher nerve. Six months after the grafting they were perfused and blocks from proximal and distal nerve as well as from the transplant were examined using phase and electron microscopy.

One month after the operation the twitcher nerve transplant seemed to be well vascularized; many axons were myelinated and there was no sign of demyelination; Schwann cells contained no inclusions but some had abnormally watery cytoplasm with scattered organelles and the endoneurial space was greater than normal. No macrophages or inflammatory cells were seen in or around the graft. In the distal stump, axonal regeneration and remyelination followed the normal expected pattern throughout the experiment. In the transplant, endoneurial oedema was more pronounced at 2 months (Fig. 2); some fibres showed early demyelination and numerous axons were surrounded by only a Schwann cell containing myelin debris; abnormally thin myelin

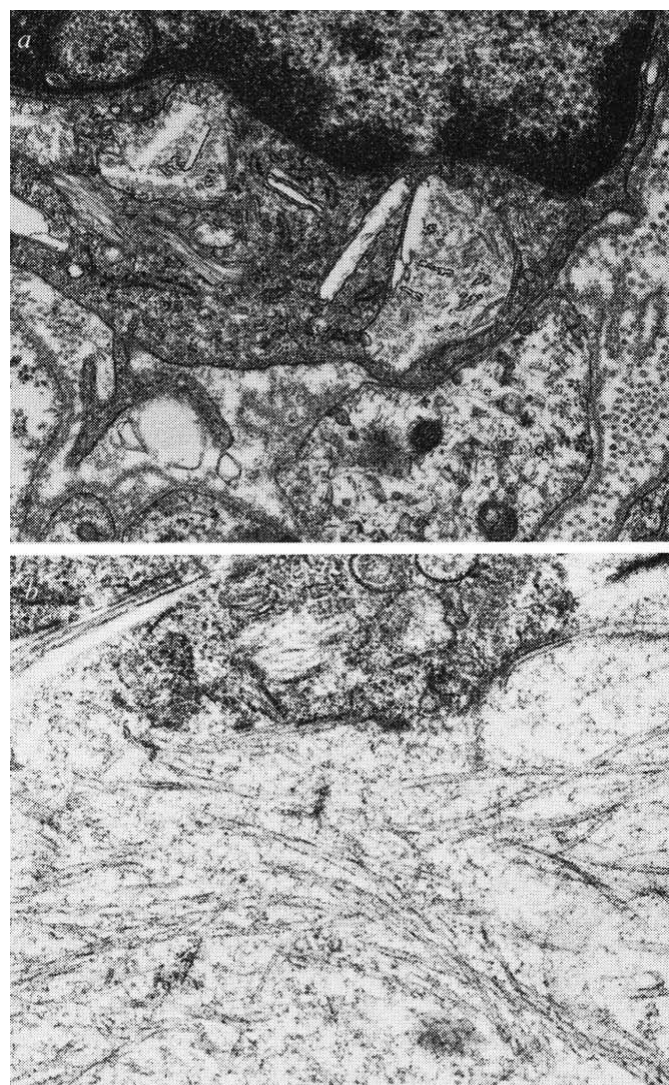


Fig. 1 Electron micrographs of macrophages containing *a*, multi-angular bodies and *b*, tubules, some of which appear twisted. Similar inclusions are also found in human and canine globoid cell leukodystrophy and are considered to be characteristic of the disease. *a* × 19,000; *b* × 36,000.

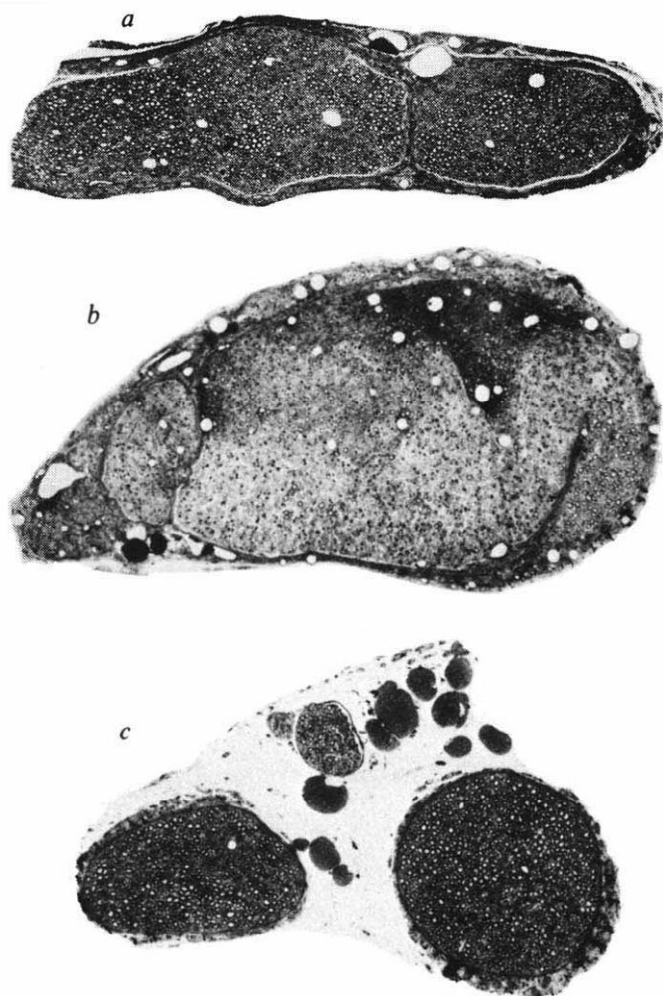


Fig. 2 One- μ m Araldite sections of: *a*, the normal proximal region; *b*, the twitcher graft; and *c*, the distal stump 2 months after transplantation. The graft is almost double the size of the proximal nerve due to the increased cellularity, severe endoneurial oedema and abundant collagen. *a*, *b*, *c* $\times 90$.

sheaths were indicative of remyelination (Fig. 3*a*, *b*). Twisted tubules, multi-angular bodies and other inclusions were seen in Schwann cells and macrophages.

At 3, 4 and 5 months some demyelinated axons were still present, but most were surrounded by apparently normal myelin and only a few Schwann cells contained myelin debris and specific inclusions. At 6 months many fibres appeared normal although the myelin sheath thickness was not comparable with the fibres in the normal distal stump. However, at each interval some features characteristic of the twitcher were still present; these comprised specific inclusions in Schwann cells and in macrophages, an occasional demyelinated axon and some endoneurial oedema (Fig. 4*a*, *b*). Autoradiographic studies confirmed the presence of labelled Schwann cell nuclei in the distal stump but no labelled cells were found in the transplant. The normal nerve grafted into the twitcher sciatic nerve showed the opposite trend, with larger myelinated fibres in the transplant than in the proximal or distal stump. These grafted nerve fibres appeared quite normal and there was no evidence of any of the other pathological features found in the twitcher host.

These experiments show that a twitcher nerve transplanted into a normal recipient reproduces all the characteristic features of leukodystrophy and, conversely, that Schwann cells from unaffected mice can produce normal myelin when associated with twitcher axons. It can be concluded, as in analogous studies by Aguayo *et al.*^{2,3}, that donor Schwann cells are able to

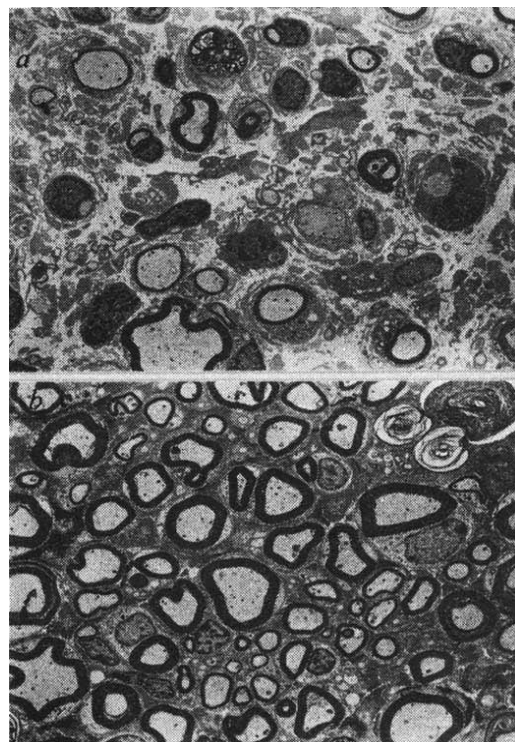


Fig. 3 Electron micrographs showing *a*, the transplanted twitcher nerve and *b*, the distal stump after 2 months. In the twitcher nerve, one fibre is demyelinating and several are completely demyelinated. In a few fibres myelin appears normal. Fibres are widely separated by endoneurial oedema, excess collagen and macrophages. $\times 1,500$.

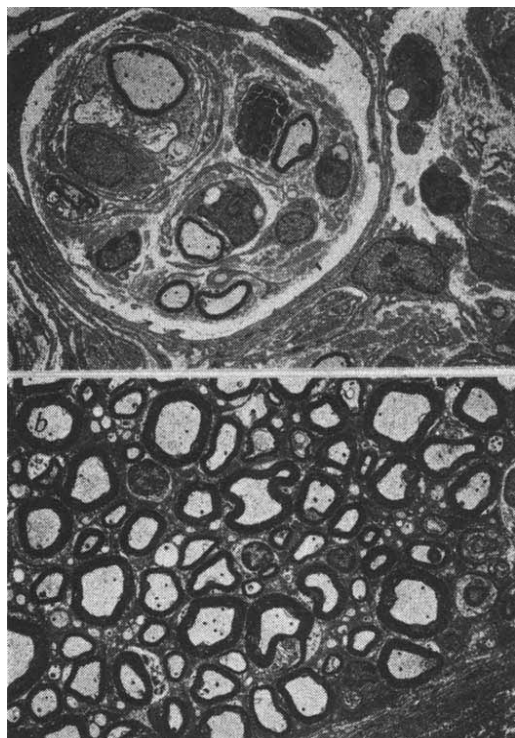


Fig. 4 Electron micrographs showing *a*, the transplanted twitcher nerve and *b*, the distal stump after 6 months. Several myelinated fibres are seen with sheaths thinner than those in the distal stump. Also visible are several inclusions in Schwann cells, a demyelinating and a thinly remyelinating fibre. The structures are more widely separated than those in the distal stump. $\times 1,500$.

reproduce an abnormality in a normal host, in association with normal axons. The twitcher axon has no apparent role in the development of the myelin sheath abnormality. Repopulation of the graft by Schwann cells from the host can be excluded on three grounds: (1) even after 6 months some specific abnormalities and abnormal myelin sheath parameters were found in the twitcher nerve grafted into a normal nerve; (2) there were neither specific inclusions nor abnormal Schwann cells and macrophages in the 37-day normal nerve transplanted into the twitcher; (3) there was no autoradiographic evidence of Schwann cell migration.

A puzzling feature was the apparent improvement towards a more normal situation in the 3–6-month grafts, which raises the question of the possible entry into the graft of enzyme from the normal host. A transitory improvement has been claimed by some authors in cases of Gaucher's disease (another enzyme deficiency condition) after transplantation of a normal spleen¹¹ and kidney¹².

The absence of rejection of the transplant without immunosuppressive treatment is unusual and does not agree with previous findings^{2–4}. It is known that a nerve transplant kept viable by anti-lymphocyte serum is quickly rejected when treatment is withdrawn³. The twitcher gene is maintained in a non-inbred strain¹ and tolerance of grafts would not be expected. The number of successful grafts in our experiment was too high to be accounted for by the random selection of immunologically matched littermates. Furthermore, a hypothetical immunological deficit in homozygous twitchers would not explain why littermates too, accept the grafts. It is possible that, although the close kinship plays a part, the non-inbred strain in which twitcher is maintained is of low immunological responsiveness¹³.

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Functional nuclei of LPS-nonresponder C3H/HeJ mice after transfer into LPS-responder C3H/HeN cells by cell fusion

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Splenic lymphocytes of C3H/HeJ mice are defective in responding to the mitogenic activity of bacterial lipopolysaccharide (LPS)^{1–4}, which has been attributed to a mutation at a single gene locus on chromosome 4 (ref. 5). It was suggested that C3H/HeJ mice lack a cell-surface receptor necessary for LPS recognition^{6,7}. However, specific binding of ¹²⁵I-labelled lipid-A of LPS to B cells was found to be equivalent in LPS-responder and LPS-nonresponder C3H/HeJ, C57BL/10 ScCR and C57BL/10 ScN mice⁸. This suggests that the genetic defect in C3H/HeJ mice is related to triggering mechanisms rather than to LPS binding. We recently established a method for separating mouse splenic lymphocytes into karyoplasts (minicells) and cytoplasts (enucleated cells) with high purity⁹. This method makes possible the exchange of nuclei from one type of lymphocyte to another. In the present study, karyoplasts were purified from LPS-nonresponder C3H/HeJ mice and transferred to mitomycin C-treated or X ray-irradiated splenic lymphocytes taken from LPS-responder C3H/HeN mice by fusion with polyethyleneglycol. These reconstituted hybrid cells could respond to LPS, suggesting that nuclei from C3H/HeJ mice could be activated by LPS if proper signals were provided from cell-surface receptors. Moreover, responsiveness to concanavalin A (Con A) of mitomycin C-treated or X ray-irradiated lymphocytes was also recovered by the microinjection of karyoplasts from untreated lymphocytes.

Cytochalasin B is known to induce enucleation of cell monolayers through centrifugation without inhibition of protein, RNA or DNA synthesis. Furthermore, the effects of this

drug on the cell mobility, cell form or permeability are readily reversible if it is removed. Enucleation can also be carried out with mouse L cells in suspension by centrifugation in Ficoll density gradients containing cytochalasin B¹⁰. We applied the enucleation method with Ficoll density gradient centrifugation to normal murine lymphocytes (see Table 1 legend; ref. 9). Enucleation occurred almost completely after centrifugation of splenic lymphocytes in Ficoll gradients containing cytochalasin B. Karyoplasts (minicells) were further purified by 1g velocity sedimentation in gradients of bovine serum albumin. After these procedures, staining with May-Giemsa's solution revealed that contamination of cytoplasts in this fraction was <1% and no intact cells were observed. Purity of karyoplasts was also examined by measuring protein and DNA contents as well as synthesis of protein, RNA and DNA after stimulation with mitogen (LPS, Con A).

In the present study, karyoplasts were prepared from LPS-nonresponder C3H/HeJ (HeJ) mice by the above method and fused to mitomycin C-treated spleen cells of responder strain C3H/HeN (HeN). Fusion was achieved with 50% polyethylene glycol containing poly-L-arginine. The addition of poly-L-arginine greatly enhanced the fusion efficiency and ~10–20% of splenic lymphocytes were found to contain two nuclei. Viability of cells was the same before and after fusion. Neither spleen cells nor karyoplasts obtained from HeJ mice responded to LPS in the range 1–100 µg ml⁻¹ (Table 1). Mitomycin C-treated HeN spleen cells fused with HeJ karyoplasts showed a strong DNA synthetic response to LPS whereas mitomycin C-treated cells or HeJ karyoplasts did not. Peak responses to LPS were seen on day 3 with fused and intact cells (data not shown). Mitomycin C-treated HeJ cells fused with HeJ karyoplasts showed no LPS mitogenic response, but they responded to Con A. In simple mixtures of mitomycin C-treated HeN spleen cells and HeJ karyoplasts, LPS responses were not observed.

In subsequent experiments, we used irradiated (1,200 rad) spleen cells as recipients instead of mitomycin C-treated cells—similar results were obtained (Fig. 1). Reconstitution of irradiated HeN spleen cells with HeJ nuclei by polyethylene glycol-mediated fusion resulted in a significant DNA synthetic response to LPS. This did not occur when HeJ karyoplasts were

inactivated by irradiation before transfer into irradiated HeN cells, suggesting that DNA synthesis in the fused cells was due to the injected HeJ nuclei rather than the host HeN cell nuclei. Fusion of nontreated HeJ cells with HeN karyoplasts did not restore LPS responsiveness although such cells showed strong responses to Con A. These data suggest that microinjection of nuclei into cells whose own nuclei are inactivated restores DNA synthetic responses to various mitogens and that the nuclei of nonresponder strain HeJ can respond normally to LPS if stimulatory signals from cell-surface receptors are appropriate.

The C3H/HeJ defect in LPS responsiveness has been attributed to a mutation of a single gene on chromosome 4 which maps close to the Mup-1 gene¹¹. Two contradictory explanations of this abnormality have been suggested—one that the mice lack suitable cell-surface receptor which is deficient in all LPS-nonresponder strains^{6,7} and the other that the LPS triggering signal(s) is not properly transferred from cell-surface receptors to the nuclei. The latter hypothesis is based on the findings that ¹²⁵I-labelled lipid-A of LPS bound equally well to the cell surfaces of responder and nonresponder mice⁸. Another possibility is that abnormal events occur in the nucleus in response to this particular mitogen in C3H/HeJ mice. In the present study we found that LPS nonresponsiveness is attributable to cell-surface or cytoplasmic events and that nuclei are functionally intact in this nonresponder strain.

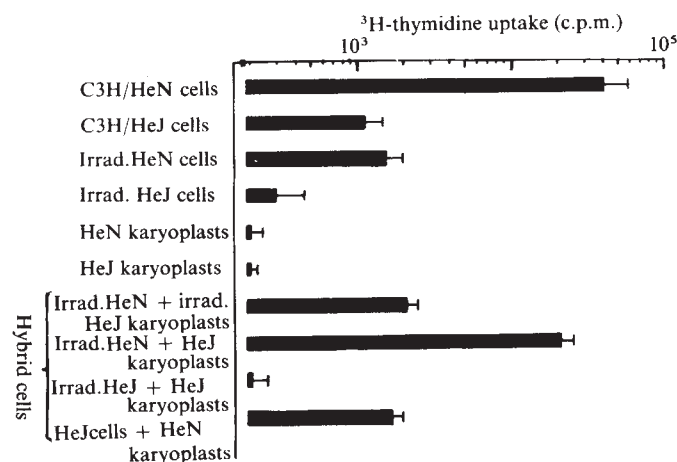


Fig. 1. Restoration of LPS responsiveness of irradiated C3H/HeN spleen cells by cell fusion. Preparations were made as described in Table 1 legend except that HeN or HeJ cells were inactivated by X-ray irradiation (1,200 rad) before fusion.

Table 1 Restoration of mitogenic response in mytomycin C-treated C3H/HeN (HeN) spleen cells after fusion with karyoplasts obtained from C3H/HeJ (HeJ) spleen cells

Responding cells	Mitogen		
	None	LPS (50 µg ml ⁻¹)	Con A (5 µg ml ⁻¹)
C3H/HeN	5,653 ± 1,122*	98,570 ± 12,682	34,193 ± 7,563
C3H/HeJ	6,025 ± 1,002	4,796 ± 310	93,029 ± 9,694
MMC-C3H/HeN	320 ± 135	1,804 ± 85	60 ± 3
MMC-C3H/HeJ	337 ± 196	254 ± 48	107 ± 38
C3H/HeJ karyoplasts	193 ± 28	218 ± 28	185 ± 11
Hybrid cells			
MMC-HeN + HeJ karyoplasts	6,586 ± 1,232	72,749 ± 8,738	57,164 ± 5,267
MMC-HeJ + HeJ karyoplasts	944 ± 152	737 ± 111	54,828 ± 20,383

Mouse spleen cells (1×10^8 cells, viability >90%) treated with ammonium chloride to remove erythrocytes were suspended in 3 ml of Eagle's minimal essential medium (MEM) and layered on Ficoll-400 (Pharmacia) gradients (discontinuous gradients of 12.5, 15, 16, 17 and 25%) in Eagle's MEM containing $10 \mu\text{g ml}^{-1}$ of cytochalasin B (Aldrich Biochemicals) and 0.5% dimethyl sulphoxide (Sigma) in cellulose nitrate tubes. Ficoll gradients were sterilized by autoclaving. Cells were centrifuged for 70 min at 23,000 r.p.m. at 31 °C with a Beckman SW-27 swinging bucket rotor in L5-65B ultracentrifuge. After centrifugation, cells in the 17–25% Ficoll fractions were collected and washed twice with 10 vol of MEM by centrifugation at 2,000 r.p.m. for 5 min. More than 95% of the cells in this fraction were karyoplasts and these were further purified by 1g velocity sedimentation using bovine serum albumin gradients (0.5–2% in phosphate-buffered saline). After fractionation with 1g sedimentation, highly purified karyoplasts were obtained (>99% of cells were karyoplasts). Spleen cells were treated with $40 \mu\text{g ml}^{-1}$ of mitomycin C (MMC) for 40 min at 37 °C before cell fusion or culture. MMC-treated spleen cells ($1 \times 10^6 \text{ ml}^{-1}$) were mixed with karyoplasts ($1 \times 10^7 \text{ ml}^{-1}$) in Eagle's MEM. The mixtures were centrifuged for 5 min at 1,500 r.p.m. in conical tubes (Falcon no. 2095), the supernatant thoroughly removed, and three drops of 50% polyethylene glycol (Koch-light MW 6,000) in Eagle's MEM containing 10% dimethyl sulphoxide and $5 \mu\text{g ml}^{-1}$ of poly-L-arginine (Sigma) were added to the cell pellet. The tubes were gently rotated for 1 min at room temperature and the reaction stopped by adding 10 ml of pre-warmed Eagle's MEM. Cells were washed with MEM at room temperature and suspended in RPMI-1640 medium containing 10% fetal calf serum and 2 mM L-glutamine. Spleen cells (2×10^5 per well), karyoplasts (1×10^6 per well) or cells containing the hybrids (2×10^5 per well) were cultured in the presence or absence of mitogen for 72 h and pulsed with $0.5 \mu\text{Ci}$ of ³H-thymidine for the last 4 h. Bacterial LPS was *Salmonella typhosa* W901 (Difco); Con A was from Sigma. Simple mixtures of MMC-treated HeN spleen cells and HeJ karyoplasts showed only marginal responses to LPS or Con A. MMC-treated HeN cells treated with polyethylene glycol in the same manner did not respond to the above mitogen (data not shown).

* Mean ± s.d. (c.p.m.).

normal lymphocytes with karyoplasts. This simple method for transferring intact nuclei from lymphocytes to inactivated recipient cells could be useful for studying subcellular events in lymphoid differentiation and contribute to our understanding of certain immunological disorders.

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B-cell activation by helper cells is a two-step process

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Recent methods for assaying helper activity and cooperative induction of B cells allow for the specific interactions of large populations of lymphocytes in culture^{1,2}. These assays, which do not select for combining-site specificity of the responding B cells, were used to investigate the precise roles of direct cellular recognition and soluble mediators in the process by which specific T-helper cells stimulate resting B cells into clonal expansion and maturation to immunoglobulin secretion. We report here results demonstrating that helper cells perform two distinct functions in the process of B-cell activation: (1) on direct recognition of antigen (and restricting elements) on B-cell surfaces, helper cells induce, in a short time, target B-cell reactivity to soluble growth factors; (2) on functional recognition of antigen on 'stimulator' cells, helper cells also participate in the production of nonspecific growth factors competent to induce proliferation and maturation in those B cells that have been 'activated' by direct interaction. Either of these two steps alone is not sufficient to induce proliferation and maturation of resting B lymphocytes.

Co-culture of helper T cells specific for B-cell surface antigens with 'target' spleen cells leads to activation of large numbers of B cells that can be detected in a polyclonal plaque assay¹. Antigenic spleen cells function in this assay as 'stimulators' of helper cell activation and, in addition, the B-cell compartment of such stimulators initiates clonal expansion and maturation. As the stimulator function of spleen cells, in contrast to the B-cell responses, is radiation resistant², 'bystander' experiments can be performed to determine whether or not B-cell activation by helper cells requires direct cell-to-cell contact. Figure 1 shows the results of an experiment demonstrating that bystander, resting B cells are not activated. C3H/HeJ helper cells specific for 'minor' antigens of the C3H/Tif substrain¹ activate C3H B cells very efficiently but fail to respond to, or to activate, spleen cells of the H-2-compatible B10.Br strain. When the two types of target cells are mixed in the same culture, in which helper-cell responses are ensured by irradiated stimulator cells³, B10.Br B cells are not activated at any effector helper/target B-cell ratio. From this finding, which has also been obtained in a completely syngeneic system using hapten-specific helper cells⁴, we conclude that successful collaboration requires direct T-B-cell interactions.

The apparent requirement for direct cell interactions in cooperative B-cell activation was difficult to reconcile with the many reports that soluble helper factors are released on T-cell activation, as these would be expected to be generated *in situ* by T-cell recognition of the appropriate stimulator cells. The above results indicate that such factors are not competent to initiate the response of resting B cells.

Schreier *et al.* have recently reported that media conditioned by activated helper cells can maintain mitogen-derived blasts, but not resting B cells, in exponential growth⁵. To assess the generation of such B-cell growth factors in our cultures, we repeated the above experiments using lipopolysaccharide (LPS)-activated B-cell blasts as bystander cells. As shown in Fig. 2, activation of helper cells by the irradiated stimulator cells

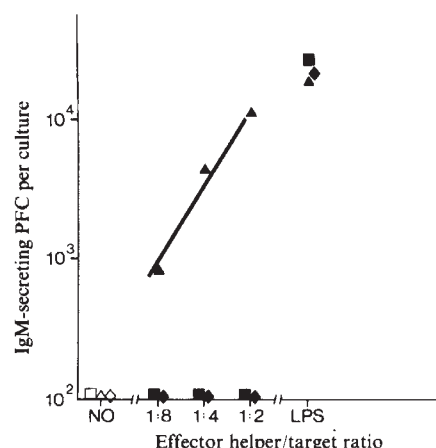


Fig. 1 The helper activity of C3H/HeJ anti-C3H/Tif helper cells, prepared as described elsewhere¹ by *in vivo* priming and *in vitro* enrichment for 4 weeks, was tested by mixing the indicated numbers with 3×10^4 target C3H/Tif (▲) spleen cells in 0.2-ml cultures in RPMI-1640 medium supplemented with 2-mercaptoethanol and 10% fetal calf serum¹. In parallel cultures helper cells were titrated into mixtures of 3×10^4 bystander B10.Br small spleen cells purified by density gradient centrifugation, with the same numbers of irradiated (3,300 rad) C3H/Tif spleen cells (◆). The IgM-secreting plaque-forming cell (PFC) responses were determined in the protein A plaque assay²⁴ 5 days later. Control responses of the same spleen cells to the B-cell mitogen LPS are also shown. Helper cells incubated with resting B10.Br spleen cells alone induce no response (■)¹.

does result in the generation of factors that maintain exponential growth and maturation in unrelated B-cell blasts.

Taken together, these results suggest that helper T cells, in addition to their role in the production of soluble nonspecific growth factors, also induce in resting B lymphocytes a state of responsiveness to those growth factors. This first step in the induction process could presumably explain the requirement for direct interactions. However, this model could only be confirmed by showing that the initial contact of resting B cells with specific helper cells could induce the 'transition' in the reactivity of the responding B cells to growth factors. In fact, the experiments with mitogen-derived B-cell blasts give no information on the initiation of helper cell-dependent induction.

To investigate these possibilities, trinitrophenyl (TNP)-derivatized BALB/c spleen cells were incubated for 48 h with specific BALB/c anti-TNP-BALB helper cells². These cultures were then depleted of T cells and the B-cell blasts originating from this initial T-B-cell interaction were purified and added as bystander cells to cultures where B10.Br anti-NP-B10.Br helper cells were stimulated by the appropriate (NP-self) irradiated spleen cells. As shown in Fig. 3, B-cell blasts activated by the cooperative interaction maintain exponential growth and maturation if unrelated T-helper cell activity takes place in the same culture. Bystander blast responses, however, strictly require concomitant, though unrelated, activation of helper cells, as there is no growth in cultures where all these cell populations were also present but the irradiated B10.Br stimulator cells were not hapten-derivatized. This control excludes 'background' activation of blasts, as well as direct interaction of NP-specific B10.Br helper cells with the BALB/c B-cell blasts. Our results demonstrate that initial T-B-cell interactions result in the acquisition of responsiveness to soluble nonspecific growth factors by the activated B cells. In addition, they show that B cells 'poised' to respond require additional soluble factors to initiate and/or maintain exponential growth.

Cooperative B-cell responses can therefore be separated into two distinct phases of which only the initiation requires direct contact with specific helper cells, whereas expansion is mediated

by soluble B-cell growth factors generated independently by helper cell interaction with competent antigen-presenting cells. It remains to be investigated whether the same helper cell is competent at the two different response levels, or whether functionally distinct T-cell subsets are involved in the initiation and expansion of B-cell responses. The cellular origin of B-cell growth factors, and their molecular characteristics are also unknown. On the other hand, previous experiments in this system have confirmed, with one exception³, the strict major histocompatibility complex restriction of the direct helper cell-B cell interactions⁴, regardless of the nature of the B-cell antigens recognized. It seems, therefore, that I-A- or I-E (C)-encoded⁶ structures on B-cell surfaces are the receptors for the helper cell signals resulting in the transition to responsiveness to B-cell growth factor. As the present experiments were performed in the absence of antigen recognition by the responding B cells whereas activation required specific antigen recognition by helper cells on B-cell surfaces, we suggest that the only role of antigen recognition (immunoglobulin-receptor binding) by B cells is that of making the antigen available on specific B-cell surfaces for T-helper cell recognition, thereby ensuring direct interaction and Ia-mediated induction⁷. These results agree with those demonstrating the necessity for specific helper cells and the failure to substitute for them with soluble factors in the response to protein antigens^{8,9}.

Classical observations, such as the requirements for 'linked recognition' of hapten-carrier conjugates and for the presence of more than one antigenic determinant in immunogenic molecules^{10,11} as well as the unidirectionality of the allogeneic effect *in vivo*^{12,13} can also be explained by this model. More recent results demonstrating the I restriction of T-B-cell interactions^{14,15} and the tendency to 'monogamy' of specific helper cells¹⁶ also fit in with the present schematic framework.

In contrast, the classical bystander experiments of Hartmann¹⁷, Schimpl and Wecker¹⁸ and Dutton *et al.*¹⁹ are in apparent contradiction with the present model. As pointed out by others^{8,9}, however, the *in vitro* responses to sheep erythrocytes are the exception rather than the rule in their lack of requirement for specific helper cells. *In vivo* experiments show no bystander effects and the responses require direct recognition of antigen by helper cells on B-cell surfaces^{15,20}. The *in vitro*

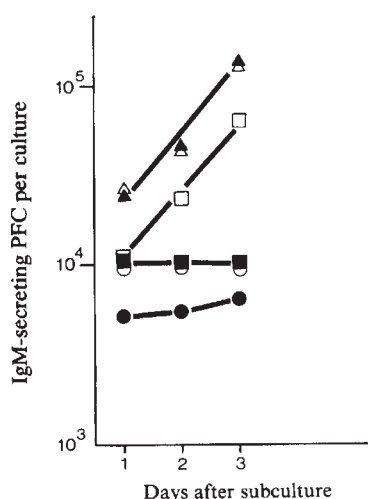


Fig. 2 B10.Br in B-cell blasts, purified by density gradient centrifugation²⁵ after 48 h of LPS stimulation were recultured (3×10^4 per culture) either alone (filled symbols) or with 3×10^4 irradiated (3,300 rad) C3H/Tif spleen cells (open symbols). Both types of cultures were stimulated with either LPS (Δ , $\blacktriangle$) 10^4 C3H/HeJ anti-C3H/Tif helper cells ($\square$, $\blacksquare$), prepared as described in Fig. 1 legend, or left untreated ($\circ$, $\bullet$). The IgM PFC responses were determined at the indicated times.

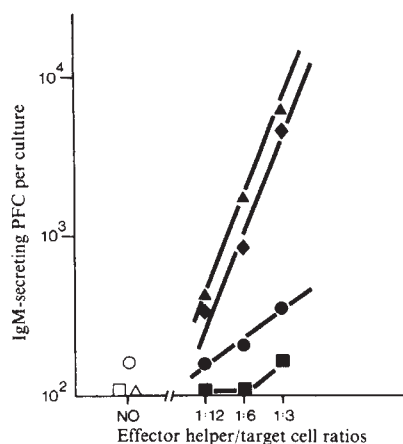


Fig. 3 Specific BALB/c anti-TNP BALB/c and B10.Br anti-TNP B10.Br helper cells used in these experiments were obtained as summarized in Fig. 1 legend, and previously described in detail, by immunization with BALB/c spleen cells derivatized with TNP or B10.Br spleen cells coupled with NP², respectively. In a first culture, TNP-derivatized BALB/c small spleen cells, purified by density gradient centrifugation, were incubated with BALB/c anti-TNP BALB/c-specific helper cells at a 3:1 ratio. After 48 h, B-cell blasts were isolated from this culture by density gradient centrifugation, followed by treatment with anti-Thy-1 antibodies and complement, and used in the test cultures shown. These contained B10.Br anti-B10.Br-NP helper cells that were cultured at the indicated concentrations with 3×10^4 non-irradiated, non-derivatized ($\blacksquare$) or NP-derivatized ($\blacktriangle$) B10.Br spleen cells. Parallel cultures of helper cells were stimulated with the same number of irradiated (3,300 rad) B10.Br spleen cells, either normal ($\bullet$) or NP-derivatized ($\diamond$) and also received 3×10^4 purified BALB/c B-cell blasts isolated from the first culture. The IgM PFC responses were measured on day 4 of culture. Open symbols represent numbers of IgM PFC observed in each type of spleen cell culture, in the absence of helper cells. No PFC responses were observed in mixed cultures of helper cells with irradiated B10.Br spleen cells, either normal or NP-derivatized (not shown).

bystander effects can be made compatible with the present model by attributing the exceptional behaviour of sheep erythrocytes to their ability to induce, in resting B cells, the transition in reactivity to growth factors²¹⁻²³.

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Clones of helper T cells mediate antigen-specific, H-2-restricted DTH

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It is now well established that in the mouse, helper T cells and killer T cells are two distinct thymus-derived lymphocyte subpopulations, differing from each other in Lyt phenotype and H-2 restriction, among other parameters. Helper T cells are Lyt-1⁺ and their action in immune responses involves restriction at the H-2I region of the major histocompatibility complex (MHC)^{1,2}. Killer cells, on the other hand, are Lyt-23⁺ and their activity is restricted by H-2K/D (refs 1, 3). In most instances, T cells mediating delayed-type hypersensitivity (DTH) responses share the Lyt phenotype and H-2 restriction of the helper T cell⁴⁻⁷. This raises the question of whether or not helper activity and DTH can be mediated by the same activated T cell. Arguments for both views have been reported⁸⁻¹². We analysed this question using clones of specific helper T cells, which were obtained by long-term culture *in vitro* of *in vivo* primed T cells, followed by single-cell cloning¹³⁻¹⁵. Here we show that these clones of helper T cells mediate antigen-specific and fully H-2-restricted DTH. The restricting element lies to the left of the I-B region in genetic maps of the mouse MHC.

Long-term cultured, cloned and subcloned sheep or horse red blood cell-specific helper T cells (T_{SR}, T_{HRC} respectively), which have been maintained for 5-24 months in culture and previously shown to function as helper T cells *in vitro*¹³⁻¹⁵ and *in vivo*¹⁵, were examined for DTH activity. This DTH responsiveness was assayed by means of the immune lymphocyte transfer assay¹⁶. As few as 1,000 cloned helper T cells, injected together with SRC into syngeneic recipients, caused a significant DTH response which increased in severity as the dose of T cells increased (Fig. 1). In comparison with *in vivo* activated lymph node cells, the cloned helper T cells were far more effective on a per cell basis. At least 10³ times more *in vivo* activated cells were required to induce a response of similar magnitude (Fig. 1, right). As controls, the other footpad of the mice was injected with cloned helper T cells without antigen or with an irrelevant antigen—in neither case was a DTH response induced. DTH responses were mediated equally well by other clones of T_{SR} and T_{HRC} and by four different clones of egg albumin-specific helper T cells when injected together with the relevant antigen.

The H-2 restriction of cellular interactions involved in DTH responses¹⁷ was examined using a clone of T_{SR} and a subclone of T_{HRC}. Absolute H-2 restriction was observed in both instances. Results obtained with T_{SR} (clone 26-14) are shown in Fig. 2. The C57BL/6J-derived cloned helper T cells mediated DTH responses only in the C57BL/6J and B10.A(5R) recipients. B10.HTT mice (incompatible with C57BL/6J mice for the whole H-2 complex) and B10.A(4R) mice (incompatible at H-2K and I-A) did not show any DTH reactivity on injection of the cloned helper T cells plus antigen. It should be stressed that this result was found after transfer of 3.3 × 10⁴ viable T cells, which is about 30 times the number of cells required to induce a significant response in a syngeneic combination. This result shows that the T cell-macrophage interactions which underlie

these DTH responses¹⁷ are restricted by either H-2K or I-A. Appropriate congenic strains were not available at the time of these experiments for distinguishing between K- or I-A-region restriction. However, the action of helper T cells in immune responses *in vivo* has been demonstrated to involve restriction only at I-A².

The antigen-specific and H-2-restricted induction of DTH response with cloned helper T cells in normal mice does not exclude the participation of host T cells at the effector level. We have therefore tested the DTH response induced by cloned helper T cells in congenic *nu/nu* mice (Fig. 2). The DTH response in these T cell-deficient animals showed the same kinetics as and was of similar magnitude to that observed in thymus-bearing, I-A-compatible mice. This result indicates that host T cells are not essential for the response and that the cloned helper T cells are the DTH effector cells.

Many investigations have attempted to establish the relationship between helper T cells and DTH effector cells⁸⁻¹². Although some studies pointed to an inverse relationship between the *in vivo* occurrence of activated helper T cells and DTH effector cells⁸⁻¹⁰, others have found that helper activity and DTH can coincide^{11,12}. These experiments were carried out with uncloned T-cell populations and failed to prove that both responses can be mediated by the same cell. However, our experiments show unequivocally that helper T cells can mediate DTH responses which were observed not only in the local immune lymphocyte transfer assay, but also after intravenous injection of these cells. Mice infused with 1-3 × 10⁶ cloned helper T cells responded to antigen challenge to the hind footpad with a smaller but significant DTH response. This requirement for much higher numbers of cells may be due to changes in recirculation and

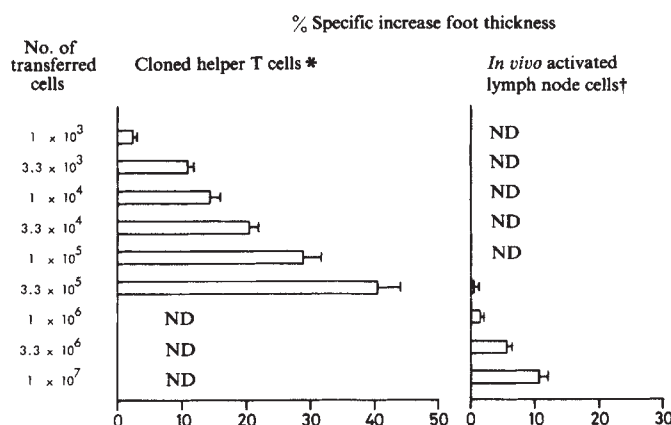
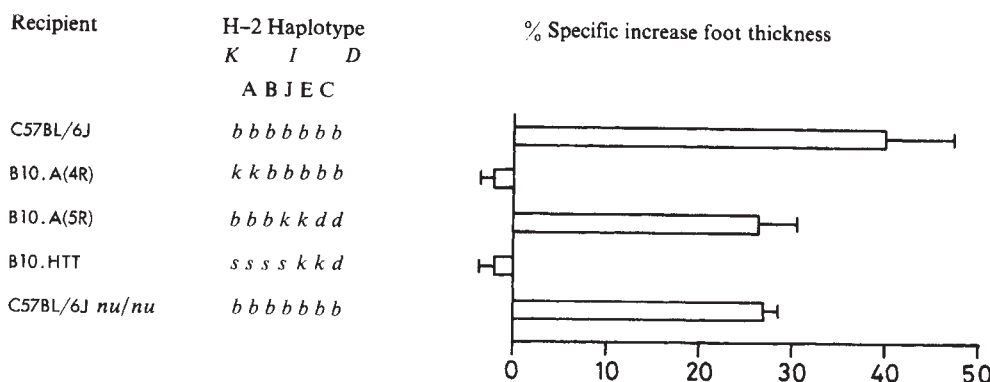


Fig. 1 DTH sensitivity by different numbers of long-term cultured C57BL/6J-derived, SRC-specific helper T cells (clone S26-14) (left), and by different numbers of lymph node cells which were activated *in vivo* by subcutaneous immunization of C57BL/6J mice with 2 × 10⁷ SRC (right). The cells to be tested were injected together with 5 × 10⁷ SRC in a volume of 50 µl into the instep of the right hind leg of C57BL/6J female mice (Institut für Biologisch-Medizinische Forschung AG). Recipient mice injected with cloned SRC-specific helper T cells plus SRC in the right hind leg received the same number of HRC-specific helper T cells (subclone 18-33-3) together with 5 × 10⁷ SRC in the left hind leg. Recipient mice which received *in vivo* activated lymph node cells plus SRC in the right hind leg received the same number of non-activated lymph node cells and 5 × 10⁷ SRC in the left hind leg. At 24 h after injection the thickness of the hind feet was measured. DTH responses are expressed as the specific % increase in foot thickness, and were calculated as (thickness right foot - thickness left foot)/thickness left foot × 100%. Horizontal bars represent 1 s.e.m. (n = 7). ND, not determined. *, The establishment and maintenance of the long-term cultured specific helper T cells has been described previously¹³⁻¹⁵. †, The activated lymph node cells were obtained by collecting the inguinal and axillary lymph nodes of C57BL/6J mice 4 days after subcutaneous immunization with 2 × 10⁷ SRC, equally distributed over the inguinal and axillary areas. The non-activated lymph node cells were obtained from naive C57BL/6J mice.

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Fig. 2 DTH reactivity mediated by cloned SRC-specific helper T cells was tested for H-2 restriction. Recipient mice (all from the Institut für Biologisch-Medizinische Forschung AG) were injected with 3.3×10^4 SRC-specific helper T cells (clone S26-14) and 5×10^7 SRC in a volume of $50 \mu\text{l}$ in the instep of the right hind foot, and with 3.3×10^4 HRC-specific helper T cells (subclone 18-33-3) and 5×10^7 SRC in the instep of the left hind foot. At 24 h after transfer the thickness of the hind feet was measured. DTH responses are expressed as the specific percentage increase in foot thickness and calculated as described in Fig. 1 legend. Horizontal bars represent 1 s.e.m. ($n = 7$).



homing properties brought about by long-term *in vitro* propagation. Similar observations were made in clonally reconstituted *nu/nu* mice where $1-5 \times 10^6$ of the same cloned helper T cells were required to induce specific antibody responses comparable with or higher than those observed in thymus-bearing mice¹⁵.

Obviously, our results do not argue against the possibility that T cells other than helper T cells may also mediate DTH reactivity. Several studies have shown that DTH to allogeneic cells can be transferred by Ly-2⁺ cells⁷ and that contact sensitivity to dinitrofluorobenzene⁶ and DTH to non-H-2 alloantigens¹⁸ and viral antigens¹⁹ can be H-2K/D restricted. Indeed, it has been reported that T cells mediating DTH and cytotoxic T cells could not be separated by size, density or antigen-binding properties²⁰.

The clones of helper T cells used for these studies have been shown to release several biologically active mediators, the induction of which is strictly antigen specific and H-2 restricted^{13,14,21-23}. So far, factors affecting B-cell^{21,22} and T-cell¹⁴ growth and *in vitro* colony formation by granulocytic, macrophage and erythroid precursor cells²³ have been discerned. Soluble factors have been indicated in the induction of the DTH lesion²⁴. Histologically, the DTH response mediated by cloned helper T cells in *nu/nu* and normal mice was indistinguishable from other Jones-Mote-type DTH responses. The nature of the cellular infiltration strongly indicates the release of chemotactic factors. Clones of helper T cells may help to characterize these activities. In addition, the DTH response is the most rapid assay to date for screening cloned and uncloned helper T cells for specificity and H-2 restricted activity. The assay is as sensitive and reproducible as the *in vitro* assay for specific helper activity. Moreover, the cellular nature of the I-A-bearing, antigen-presenting cells may be identified by their simultaneous injection with helper T cells into I-A-incompatible hosts.

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Altered expression of the calcitonin gene associated with RNA polymorphism

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The selective control of gene expression results in diversified morphology and physiological function. Understanding the expression of differentiated function in molecular terms requires detailed characterization of the regulation of mRNA synthesis and catabolism. Although considerable emphasis has been placed on transcriptional control, the discovery of HnRNA and 'split genes' gives rise to the possibility of post-transcriptional regulation at the level of processing of nuclear precursors^{1,2}. A rat calcitonin-producing medullary thyroid carcinoma (MTC) line was used as a model for definition of certain aspects in regulation of gene expression. Serial transplantation of several rat MTC lines containing and secreting large amounts of calcitonin generated tumours in which calcitonin biosynthesis was decreased more than 10-fold. We report here that the conversion from a 'high' to a 'low' calcitonin producing state is associated with specific modifications of the calcitonin mRNA synthetic pathway and a consequence of these changes seems to be the production of a new cytoplasmic mRNA.

Variation in calcitonin biosynthesis typifies a frequent event in many hormone-producing tumours characterized by a spontaneous reduction in synthesis of a particular hormone, often in association with the appearance of new and frequently predictable hormones³. Thyroid 'C'-cell hyperplasia and ultimate development of MTCs occurs in 10–40% of older rats⁴. Production of calcitonin by these tumours may decrease spontaneously during a single transplantation or following reintroduction of primary culture cells, or gradually during serial transplantation, from the usual 1.5–4.5% of total protein to <0.3% of total protein⁵. The direction of change is characteristically from high to low calcitonin production. Immunohistochemical staining with specific calcitonin antisera reveals that ~95% of the cells in high-producer tumours and <5% of cells in low-producer tumours are calcitonin positive. The conversion to low calcitonin production is frequently accompanied by increased production of somatostatin⁶. These tumours also produce other polypeptide hormones⁷.

Characterization of rat calcitonin mRNA has allowed detailed analysis of calcitonin biosynthesis. mRNA from MTCs programmes the cell-free synthesis of a calcitonin precursor peptide of molecular weight (MW) 17,500 (ref. 8) (alternatively estimated as 15,000 (ref. 9)) and a less prevalent product of MW 18,800 (ref. 8). DNA sequence analysis of a chimaeric plasmid containing DNA complementary to calcitonin mRNA establishes that the 32-amino acid calcitonin sequence is encoded in the 3'-proximal portion of the mRNA¹⁰. Using this clone as probe, normal rat thyroid and high-producer rat MTCs

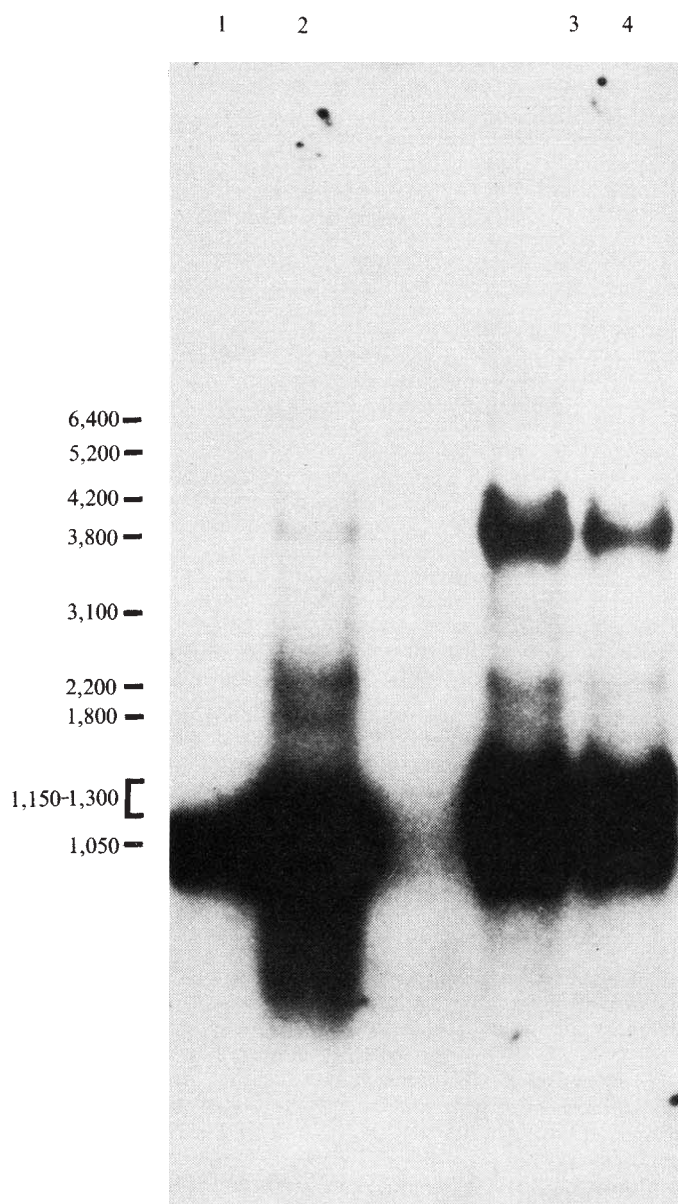


Fig. 2 Analysis of total cellular RNA species hybridizing to calcitonin cDNA. Poly(A)-rich RNA was prepared and processed as described in Fig. 1 legend. The autoradiograph was exposed for 4 days to visualize clearly the larger reactive RNA species. Lane 1, cytoplasmic poly(A)-rich RNA (5 μ g) from a high-producer tumour; lane 2, total poly(A)-rich RNA (10 μ g) from a high-producer tumour; lane 3, total poly(A)-rich RNA (10 μ g) from a low-producer tumour; and lane 4, total poly(A)-rich RNA (10 μ g) from a low-producer tumour of independent origin.

Fig. 1 Analysis of RNA species hybridizing to calcitonin cDNA. Poly(A)-rich RNA was prepared as described elsewhere^{8,11} from high- and low-producer tumours. Aliquots were denatured by reaction with glyoxal¹² and electrophoresed on 1.5% agarose slab gels using 36 mM Tris-HCl (pH 7.6), 30 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 1 mM EDTA¹³. The gel was stained using acridine orange, photographed and gently shaken for 20 min with 50 mM NaOH, then neutralized with two washes of 0.2 M sodium acetate (pH 4.3). The RNA was then transferred to diazotised paper, washed in prehybridization solution, and hybridized for 14 h at 42°C against 5×10^5 c.p.m. ml^{-1} of ^{32}P -labelled calcitonin cDNA, produced by nick-translation of calcitonin-containing plasmid to a specific activity of 1.5×10^8 c.p.m. μg^{-1} (refs 13, 14) using dCTP as the labelled nucleotide. The paper was washed and autoradiographed as previously described. The migration of 18S and 28S ribosomal RNAs and of prolactin mRNA was used to calculate size; the portion of the gel which was blotted contained 18S RNA, as shown. The 1,050-nucleotide species is referred to as calcitonin mRNA. a, Poly(A)-rich RNA from a high-producer tumour (10 μ g); b, poly(A)-rich RNA from a low-producer tumour (10 μ g).

were shown to contain one major poly(A)-rich cytoplasmic RNA species $1,050 \pm 50$ nucleotides in length¹⁰, referred to as calcitonin mRNA.

To identify mechanisms by which variation in calcitonin gene expression occurs, total poly(A)-rich RNA for high- and low-producer tumours was examined. Analyses were carried out by hybridizing labelled, cloned calcitonin cDNA to RNA transferred to diazotised paper after denaturation and size-fractionation through agarose gels (see Fig. 1 legend). In the conditions used, this method provided an accurate and reproducible quantification of RNA species which varied from 1,000 to 5,500 nucleotides in length over a 200-fold range of

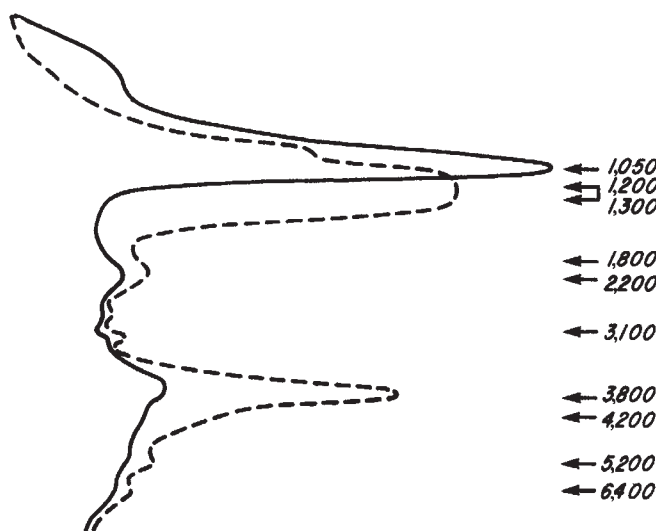


Fig. 3 Scan analysis of autoradiographs of calcitonin cDNA-reactive species. Autoradiographs of calcitonin cDNA-reactive RNA were quantified by scanning with a Helena Quickscan (1-mm window). The sizes of various RNA species are indicated, based on migration of 18S and 28S rRNA. —, High- and - - -, low-producer tumour RNA.

RNA concentration. Remarkable differences in the abundance and size of reactive poly(A)-rich RNA from low-producer tumours were observed. As shown in Fig. 1b, low-producer tumours contain a 1,050-nucleotide transcript which co-migrates with calcitonin mRNA of both high-producer tumours and normal thyroid gland, and a second abundant species which migrates at 150–250 nucleotides longer than calcitonin mRNA (Fig. 1a). Note that this second species seems to lack a similar correspondent in the high-producer lines which suggests that conversion from high to low production is associated with an altered expression of the calcitonin gene or a closely related gene.

To examine this relationship more closely, it is necessary to identify the steps involved in the synthesis of mature calcitonin mRNA. Total poly(A)-rich RNA from high- and low-producer tumours was examined for putative precursor transcripts. In addition to the prominent 1,050-nucleotide cytoplasmic species, RNA from the high-producer tumour contains several calcitonin cDNA-reactive RNA species (Fig. 2, lanes 1 and 2). These include transcripts of 6,400, 5,200, 4,200, 3,800, 3,100, 2,200 and 1,800 nucleotides in length.

Analysis of RNA from two independent low-producer tumours revealed surprising alterations in abundance of most of the nuclear transcripts. There were large increases in the 6,400-, 5,200-, 4,200- and 3,800-nucleotide species whereas the 1,800-nucleotide transcript was significantly decreased (Fig. 2, lanes 3 and 4). The abundant RNA species are overexposed on the autoradiograph (Fig. 2) to visualize the less-abundant putative nuclear RNA species.

Scans of the autoradiographs (Fig. 3) allowed quantification of reactive RNA species. The amount of the 3,800-, 5,200- and 6,400-nucleotide RNA species in low-producer tumours was increased 10- to 30-fold above that in high-producer tumours. In the low-producer tumour, the 3,800-nucleotide RNA species alone bound up to 25–30% of the total probe hybridized. There was no alteration between high- and low-producer tumours in the amount of reactive 3,100- or 2,200-nucleotide RNA species.

Conversion to a low calcitonin producer state was associated with a series of reproducible alterations in calcitonin gene expression. Cells which contained little or no detectable calcitonin transcribe large amounts of calcitonin-reactive nuclear RNA species and generate new cytoplasmic RNA species, 150–250 nucleotides larger than the 'mature' calcitonin mRNA.

The molecular alterations which occur during conversion from high to low calcitonin production could result from one of several possible mechanisms: (1) an increased transcriptional activity of a related gene (or genes), with concomitant reduced expression of the calcitonin gene, corresponding to the reduction in calcitonin synthesis; (2) an alteration in the primary structure of a single calcitonin gene, with resultant alteration in processing of the primary transcript; or (3) the observed RNA heterogeneity in low-producer tumours arises from differential or 'abnormal' processing of one primary RNA transcript. In the latter two cases, the increased abundance of the putative nuclear precursor RNAs could correspond to an altered processing pathway; any of the proposed models could result in the observed RNA polymorphism. To investigate these possibilities, a chimaeric plasmid containing DNA complementary to the additional RNA sequence unique to the new 1,250 RNA present in low-producer tumours has been constructed. This will allow initiation of structural and functional analysis of the new cytoplasmic RNA species, the putative nuclear RNA transcripts, and of the calcitonin gene.

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Dual expression of λ genes in the MOPC-315 plasmacytoma

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The expression of two distinct κ light chain immunoglobulins in the MPC-11 mouse myeloma is well established^{1–4}, the two protein products being apparently from RNA transcripts derived from separate, rearranged κ alleles in the MPC-11 genome^{5–7}. Recently, the characterization of κ -related RNAs and protein products in several λ -producing myelomas has indicated that multiple expression of light chain RNAs is a common event in myelomas and other cells of the B-lymphocyte lineage⁸. These studies suggest that, although many light chain alleles may function to make RNA and protein in a given B-lymphocytic cell, only one complete, functional light chain is generally translated from the RNAs present in a single cell. The myeloma, MOPC-315, synthesizes and secretes an antibody

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which has an α heavy chain and a λ_{II} light chain⁸. The DNA of MOPC-315 either has no κ genes or has only a fragment of one, but it certainly has no κ genes in the embryonic configuration⁵. Rearrangement of its λ genes has been observed but the exact nature of the rearrangement is not known⁹. Because initial observations suggested that an immunoglobulin-related protein other than α and λ_{II} was present in MOPC-315 cells¹⁰, we undertook to derive molecular cDNA clones from the mRNA in MOPC-315 tumour cells. Analysis of the clones has now identified two λ chain mRNA species: a normal λ_{II} chain mRNA and another which directs the synthesis of a deleted form of a λ_I protein. The nucleotide sequence of the deleted λ_I mRNA shows that it resulted from a joining of the sequence encoding amino acid 31 of the variable region directly to the constant region coding sequence.

To characterize the λ RNA and protein products in MOPC-315 we first derived cDNA clones from total poly(A)-containing RNA of the tumour. In parallel, *in vitro* translation studies on partially purified mRNAs and RNA size analysis with cloned λ probes were performed¹⁰. Clones were derived using the dG-dC homopolymer tailing method to insert DNA in the *Pst*I site of pBR322. After transformation of *Escherichia coli* χ 1776, recombinant plasmid-containing cells were selected that hybridized to a full length λ_I cDNA clone previously derived from myeloma MOPC-104E (A.L.M.B., unpublished). Preliminary restriction enzyme digest patterns indicated the presence of two types of λ cDNA clones. Two of the longest clones representative of the two classes of inserts were chosen for detailed examination. Because one was shown to have λ_{II} sequence and the other λ_I sequence, they were named $p\lambda_{II}$ -1 and $p\lambda_I$ -13.

The relationship of the clones to cellular mRNAs was studied by using the cDNA clones to select mRNAs by hybridization and

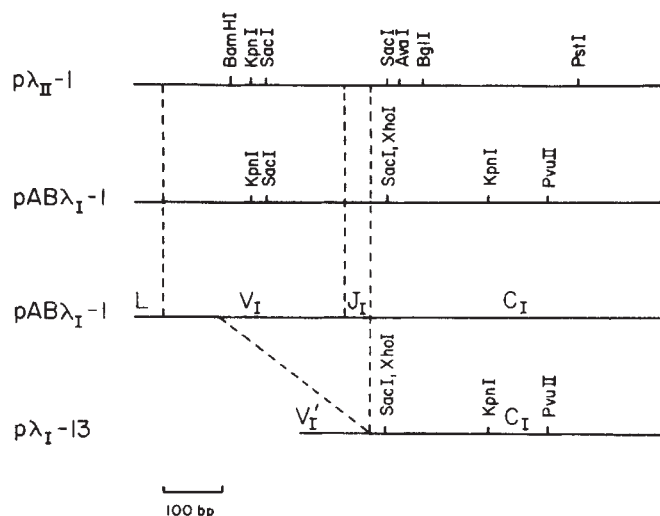


Fig. 2 Restriction maps of the λ_I and λ_{II} cDNA clones. The maps indicate the deletion of the 3'-proximal two-thirds of the variable region and J region from $p\lambda_I$ -13 when compared with the normal λ mRNA. The $pAB\lambda_I$ -1 clone (from MOPC-104E, a λ_I producer) contains a portion of the leader sequence whereas the $p\lambda_I$ -13 clone begins at amino acid 8 in the mature protein. The embryonic λ_{II} variable sequence does not contain a *Bam*HI site¹³. A different amino acid occurs at amino acid position 38 (isoleucine) in the MOPC-315 λ_{II} sequence from that predicted from the germ-line λ_{II} variable gene DNA sequence (valine)¹³. The codon ATC for residue 38 (isoleucine) would generate a *Bam*HI site which is found in $p\lambda_{II}$ -1. bp, base pairs.

then translating the mRNAs *in vitro* (Fig. 1). Two λ -related protein products could be detected by *in vitro* translation of mRNA. When the total translation products (lane 1) were submitted to specific immunoprecipitation, an 18,000 molecular weight (18K) protein was precipitated with anti- λ_I serum (lane 2) and the complete λ_{II} light chain was precipitated with anti- λ_{II} serum (lane 3). When the mRNA selected by $p\lambda_I$ -13 was translated, an 18K protein was enriched (lane 4) which was precipitable with anti- λ_I serum (lane 5) but not with anti- λ_{II} serum (lane 6). By contrast, when $p\lambda_{II}$ -1 was used to select mRNA, the selected mRNA translated into λ_{II} protein (lane 7) which was not precipitable with anti- λ_I serum (lane 8) but was precipitable with anti- λ_{II} serum (lane 9). We have independently shown that the $p\lambda_{II}$ -1 clone hybridizes to a mature size light chain mRNA (1,100 base pairs) whereas the $p\lambda_I$ -13 clone hybridizes to a mRNA of about 900 base pairs¹⁰. Thus, it is evident that MOPC-315 cells have two types of λ -related mRNA which gave rise to two classes of clone: $p\lambda_{II}$ -1 derived from a full-length mRNA encoding the λ_{II} protein of MOPC-315 antibody and $p\lambda_I$ -13 derived from a truncated λ_I mRNA that translates to form a truncated λ_I -related protein.

The $p\lambda_I$ -13 clone was further analysed by comparing its restriction enzyme digest pattern with that of a clone derived from the full-length λ_I mRNA of myeloma MOPC-104E prepared in this laboratory (A.L.M.B., unpublished) (Fig. 2). The authentic λ_I clone had six identifiable sites, two of which (*Xho*I and *Kpn*I) in the constant region would not have been predicted from the reported 138-base pair sequence¹¹. Sequence data, reported below, have confirmed the existence of these sites in this and in other λ_I cDNA clones; presumably, there were sequencing errors in the previous report. The $p\lambda_I$ -13 clone from MOPC-315 had all the sites found in the authentic λ_I constant region sequence. These distinguish the cloned insert from the λ_{II} genes, the sites of which are quite different from those in the λ_I gene (Fig. 2).

To analyse the structure of $p\lambda_I$ -13 further, we determined the nucleotide sequence of its insert (Fig. 3). The entire constant region sequence was evident but the variable region sequence contained codons that, by comparison with the reported protein

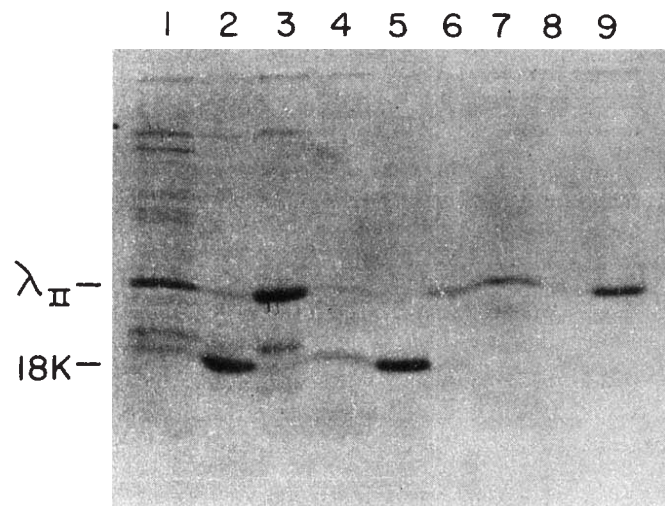


Fig. 1 *In vitro* translation of clone selected mRNA. Either an *Eco*RI-*Xho*I 1,100-base pair DNA fragment derived from $p\lambda_I$ -13 plasmid (10 μ g) or a *Pvu*II-*Ava*I 1,800-base pair DNA fragment derived from 10 μ g of $p\lambda_{II}$ -1 containing the λ_{II} constant region was denatured and bound to a nitrocellulose filter and hybridization and elution procedures performed¹⁸. Membrane-bound poly(A)-containing polysomal RNA (5 μ g) derived from MOPC-315 (tumours maintained by Dr H. Eisen) was hybridized in 65% formamide, 400 mM NaCl, 10 mM PIPES, pH 7.4, at 45 °C for 2 h. The filters were washed 10 times with 150 mM NaCl, 20 mM Tris-HCl pH 7.8, 1 mM EDTA, 0.5% SDS at 65 °C, then three times in 20 mM Tris-HCl pH 7.8, 1 mM EDTA. RNA was removed by boiling for 1 min with 300 μ l of H₂O, ethanol precipitated and translated in a rabbit reticulocyte lysate. Lanes contain total polysomal RNA translation products (lanes 1-3), $p\lambda_I$ -13-selected mRNA translation products (lanes 4-6) or $p\lambda_{II}$ -1-selected mRNA translation products (lanes 7-9). Products in lanes 2, 5 and 8 were precipitated with anti- λ_I serum and those in lanes 3, 6 and 9 with anti- λ_{II} serum. The sources of the antisera and immunoprecipitation procedures are described elsewhere¹⁰.

The possibility of an aberrant splice as the cause of the truncated λ_1 mRNA in MOPC-315 is supported by an examination of the nucleotide sequence of the λ_1 variable region. The sequence TAGTAACTA that occurs immediately 3' to the deletion site in the complete λ_1 variable region¹¹ has the form close to the canonical donor splice sequence^{14,15}. It is also clear from a comparison of the cleavage points demanded by the sequence of the cDNA clone (indicated by arrows)

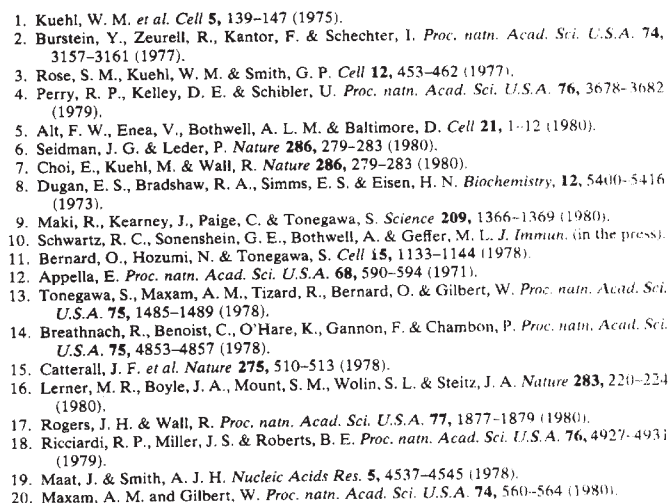


Fig. 3 Nucleotide sequence of λ I-13. The sequence of the insert was determined by both the Sanger dideoxynucleotide extension procedures according to Maat and Smith¹⁹ and the Maxam-Gilbert methods²⁰. The sequence of both strands was determined by kinase treatment at the following sites: *Pst*I, *Xho*I, *Pvu*II and *Mbo*II (at position 207). The two residues below the sequence (G, T) are only differences between the λ I and λ II variable regions in this clone. The numbers above the translation sequence are the amino acid positions in the mature protein and the numbers below the DNA sequence indicate the number of base pairs from the first nucleotide in the inserted λ I sequence as it is drawn here.

Recombination suppression of mouse *t*-haplotypes due to chromatin mismatching

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We show here that recombination occurs at the normal rate in compound female mice containing two different complementing lethal haplotypes (t^{h17}/t^{w12}) where there is a long stretch of homologous *t*-mutant chromatin. Thus the recombination suppression of a complete *t*-haplotype cannot be due to an intrinsic factor(s) which suppresses along the length of its own chromosome but is due to 'mismatching' of wild-type and mutant chromatin. Naturally occurring *t*-haplotypes of mouse chromosome 17 have several interesting genetic properties. First, they are always transmitted from males in much higher proportions than mendelian expectation; presumably this accounts for the maintenance of lethal and semilethal *t*-haplotypes at polymorphic levels in populations of wild mice. *t*-Haplotypes also show recombination suppression. The conventional map distance between genetic markers *T* and *tf* is 7–12 cM, whereas in (*t*/+) heterozygotes for naturally occurring *t*-haplotypes, recombination is suppressed and *T* and *tf* seem to be separated by only 0.1–0.5 cM (ref. 1). The region of recombination suppression extends to and includes the major histocompatibility complex (H-2)². Thus *t* and H-2 effectively travel as a single unit—a 'super gene'³. Although recombination suppression is known to be accompanied by failure of chiasma formation⁴, the mechanism underlying the suppression has remained an enigma. Lyon suggested a disorder of *t*-heterochromatin⁵ and more recently a change in 'intercalary' middle repetitive DNA⁶. She proposed that either *t*-chromatin is intrinsically incapable of participating in crossing-over, or chiasma formation is prevented because of mismatching and mispairing of normal and abnormal chromatin. We have measured recombination between two chromosomes which carried extensive overlapping segments of *t*-chromatin. We report here that in this configuration, recombination occurs at a normal rate, and thus we conclude that cross-over suppression is due to mismatching.

Rare recombination events do occur in *t*/+ heterozygotes and produce reciprocal cross-over products that are profoundly different. Whereas intact *t*-haplotypes contain a factor responsible for interaction with *T* to produce taillessness as well as a recessive lethal factor, those recombinants that retain the centromeric end of the parent *t*-haplotype continue to interact with *T* to produce taillessness, but are no longer lethal when homozygous, and those that acquire the distal end of the original *t*-chromosome retain the lethal factor but no longer show any interaction with *T*. Both cross-over products show limited recombination suppression in heterozygotes: *t*-viable haplotypes suppress it about half-way through the *T*-*tf* interval but not beyond⁷; *t*-lethal haplotypes seem to continue to be inextricably linked to H-2 although they permit free recombination between *T* and *tf*⁸.

Lyon investigated the problem of cross-over suppression by measuring recombination between recombinant chromosomes that contained, in one case, the proximal end and in the other the distal lethal factor, and which were thought to have a short overlapping region of *t*-chromatin. Using t^{h2} and t^{h17} (see Fig. 1a) she found very low recombination (1.5% compared with 18.1% expected for $t^{h17}/+$), and concluded that each *t*-haplotype suppressed recombination over its own length. She also

tested several haplotypes that are thought to be non-overlapping, and found that crossing-over was normal where the wild-type chromatins coincided (Fig. 1b, for example). These experiments did not rule out the possibility that cross-over suppression is due to mismatching of *t* and wild-type chromatin.

The characteristics of the two chromosomes we used (see Fig. 2) were as follows. The t^{h17} chromosome was derived by Mary Lyon⁸. It carries *T*; t^{h17} , an isolated lethal factor derived from t^6 (t^6 complementation group); +^{tf} and translocation 190 (T190) inseparably linked to the lethal factor. *Trans* to wild type, it allows recombination in the region of homozygous wild-type chromatin between *T* and t^{h17} which we measure in males as 16.6% (193/1,161) between *T* and *tf*. This agrees well with Lyon's figure of 18.1% for males and females⁶. The $t^{w12}tf$ chromosome is a complete lethal haplotype containing t^T (the mutant tail interaction factor), t^{w12} lethal factor (t^{w12} complementation group) and *tf*. The *tf* on this chromosome appeared spontaneously either as a point mutation or small deletion; recombination was not involved because the t^{w12} chromosome still retains its original H-2 type (Craig Hammerberg, unpublished data). We maintained the $t^{w12}tf$ chromosome in the balanced lethal cross (*Ttf/t^{w12}tf* × *Ttf/t^{w12}tf*) homozygous for *tf* and thus the recombination rate is difficult to estimate accurately (see Table 1, line 2). However, recombination from the parental t^{w12} + chromosome where the exchange of tufted can be monitored is 4.4×10^{-3} (24/5,576) (see Table 1, line 3). Taken together, the recombination rate between *T* and *tf* in the presence of $t^{w12}tf$ or t^{w12} + is <0.5%.

In choosing the two haplotypes t^{h17} and $t^{w12}tf$, we also took into consideration the fact that they share the same H-2 type, as this suggests that the two chromosomes may have a common descent from a single ancestral *t*-haplotype by mutation, and thus that their abnormal chromatins would be homologous.

Recombination measured in $t^{h17}/t^{w12}tf$ females was an extremely high 18.4% (Table 1, last cross). This is remarkably close to the value of 17–18% for t^{h17} *trans* to wild type, and high for the interval *T*-*tf*. Clearly, recombination is occurring freely between the t^{h17} and $t^{w12}tf$ chromosomes.

Of the 14 recombinant chromosomes obtained, 7 have so far been analysed. The important question was whether the two reciprocal types of recombinants, short-tailed tufted (*Ttf*) and normal-tailed non-tufted (t^T + ^{tf} T190) carried one, or both, or

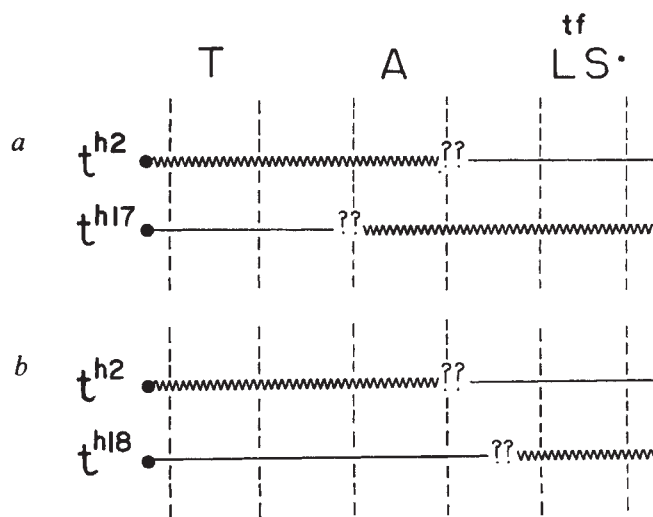


Fig. 1 Situations in which Mary Lyon measured recombination; diagrammed after Lyon *et al.*^{8,9}. T, the region of the Brachyury mutation (*T*), and the tail interacting factor (t^T) present in complete lethal *t*-haplotypes. A, as denoted by Lyon, the region of a ratio factor. LS, the region of lethality and sterility factor (*s*) is also the region where the marker tufted (*tf*) maps. a, Recombination between chromosomes with a short overlapping region of *t*-chromatin; b, recombination in haplotypes thought to be non-overlapping.

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Table 1 Recombination data for T - tf interval in t^{h17} and t^{w12} mice

Cross	Normal tailed tufted	Normal tailed non-tufted	Short tailed tufted	Short tailed non-tufted	Tailless tufted	Tailless non-tufted	Recombination rate (%)
$\delta Tt^{h17}T190 +^{tf}/++ \times \delta + + / + + tf$	405	94*	99*	563	NE	NE	16.6†
$\delta Tt^{w12}tf \times \delta Tt/t^{w12}tf$	NE	16*	NE	NE	6,682	NE	0.24
$\delta Tt/t^{w12} +^{tf} \times \delta Tt/t^{w12} +^{tf}$	NE	20*	NE	NE	4.5‡	5,576	0.44
$\delta Tt^{h17}T190 +^{tf}/t^{w12}tf \delta + + / + + tf$	27	7*	7*	35	NE	NE	18.4

Mice were scored for tail length at birth and for tufted at 28 ± 2 days. The second and third group of crosses are balanced lethals where, in the absence of recombination, the only expected phenotype at birth is tailless; T/T and t^{w12}/t^{w12} embryos die before birth. All the normal tailed mice born were genetically analysed for their recombinant chromosome. Those that were fertile all proved to be $t^{w12}/t^{viable}tf$, that is carrying the proximal end of t^{w12} haplotype with the tail interaction factor but having lost the distal lethal component. The tailless tufted mice in the third cross represent the same class of recombinant but heterozygous with $T(Tt/t^{viable}tf)$. The reciprocal recombinant, $Tt^{lethal}+$, presumably goes undetected in this cross because it cannot meet a chromosome with which it is viable. NE, not expected.

* Recombinant phenotypes in each cross.

† The numbers in this cross include previously unpublished data of L. C. Dunn and D. Bennett, 1965–1973, and current data up to 1979.

‡ The 4.5 tailless tufted is a projected number based on one tailless tufted out of 1,242 tailless mice that were raised and scored for tufted.

Table 2 Analysis of new recombinant haplotypes

Haplotype	Cross	Complementation data			Recombination data				Rate (%)
		Normal tail	Short tail	Tailless	Normal tailed non-tufted	Normal tailed tufted*	Short tailed non-tufted*	Short tailed tf	
$Tt^{s1}tf$	$\frac{Tt^{s1}}{++} \times \frac{++}{t^{s1}t^0}$	83	33	12	7	0	1	8	6.3
	$\frac{Tt^{s1}}{++} \times \frac{++}{t^{w12}}$	142	23	2†					
$Tt^{s5}tf$	$\times t^0 \frac{+}{+}$	28	36	11	21	3	5	75	7.7
	$\times t^{w12}$	21	28	0					
Tt^{s6}	$\times t^0$	12	26	16	37	1	2	54	6.1
	$\times t^{w12}$	11	29	0					
Tt^{s10}	$\times t^0$	12	6	3	41	4	6	84	7.4
	$\times t^{w12}$	27	0	0					
Tt^{s11}	$\times t^0$	15	35	14	31	5	4	54	9.6
	$\times t^{w12}$	95	15	2†					
$t^{s8}T190$	$\frac{T}{t^0} \times \frac{T}{t^0}$	0	NE	17			No data		
$t^{s8}T190$	$\frac{T}{t^{w12}} \times \frac{T}{t^{w12}}$	3	NE	2					
$t^{s9}+$	$\times t^0$		No data				No data		
	$\times t^{w12}$	5	NE	2					

The new recombinant haplotypes were arbitrarily designated $t^{s1,s2}$ and so on in order of their detection. For the complementation data, the numbers at birth contain pooled male and female data (except for $Tt^{s10} \times t^{w12}$) and are usually distorted reflecting the male ratio. Recombination data are obtained from crosses of $Tt^{s1}tf/++ \times +/++tf$. Data are pooled reciprocal crosses except in the case of Tt^{s11} where the data are from females only. The numbers scored reflect preferential culling at birth against normal tails. NE, not expected.

* Recombinant phenotypes.

† This class in Tt^{s1} and Tt^{s11} are recombinants; three tested as recombinants, one never bred.

‡ Same as for t^{s1} crosses above.

neither of the lethal factors present on the parental chromosomes. The complementation data shown in Table 2 demonstrate that all five Ttf recombinants analysed carry the t^{w12} but not the t^{h17} lethal factor.

The data for the $t^+ +^{tf}$ T190 recombinants are still sparse, as the T190 translocation renders them semi-sterile, and difficult to analyse in complementation tests. Nevertheless, available data (Table 2) suggest that this class contains the t^{h17} lethal factor but not t^{w12} . At face value these data suggest that t^{w12} and t^{h17} act as alleles. However, three alternative and equally likely interpretations must be considered. First, t^{w12} and t^{h17} may be very closely linked, and only the analysis of many recombinants could define them as genetically separate. Second, all the recombination may be taking place in a restricted 'hot spot' to the left of both t^{w12} and t^{h17} . There is some evidence from exceptional recombination in mice heterozygous for t -chromatin that the second possibility is true. In eight exceptional recombinants from balanced lethal stocks containing various complete t -haplotypes and also an interstitial marker qk between T and tf ($T(3)qk(4)tf$), all eight cross-overs were between qk and the t

lethal factor (K.A., unpublished data). In the present study another marker ($Tcp-1$), known to be between qk and the t lethal factor⁹, was monitored; 10/10 recombinant chromosomes tested resulted from cross-overs between $Tcp-1$ and t^{lethal} . A third, obvious possibility is that the lethal factors are actually located distal to $tufted$, and as we screen for recombinants using $tufted$, they will always be exchanged.

Recombination data for the new haplotypes $Tt^{s1,s5,s6,s10,s11}$ (all of the $Tt^{w12}tf$ type) are also presented in Table 2. Clearly they now all allow recombination, just as the parental haplotype t^{h17} does, in the region of homozygous wild-type chromatin between the centromere and the t^{w12} lethal factor. All nine such recombinants tested were between T and t^{w12} . Recombination in these heterozygotes, however, is about 8–11%, considerably less than that from the Tt^{h17} T190 chromosome; this supports the suggestion of Lyon⁶ that the very high recombination in the translocation heterozygotes was produced by the translocation itself.

The transmission ratio data for the various recombinant haplotypes are given in Table 3. Clearly the Tt^+ chromosomes

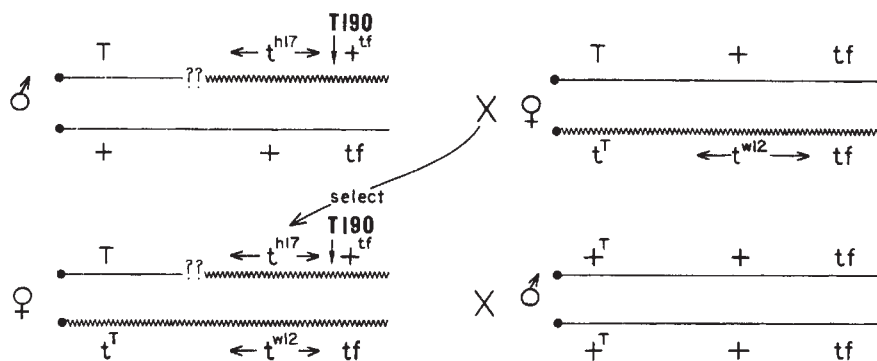


Fig. 2 Construction scheme of compound heterozygotes for t^{h17} and $t^{w12}tf$ and their expected offspring when mated to normal tail tufted. Short-tailed t^{h17} carriers were mated to tailless $t^{w12}tf$ mice. The tailless offspring, which are $Tt^{h17}/t^{w12}tf$, were selected. Males were tested for fertility. 4/4 were sterile, as expected, because they carried two complementing lethal factors. Females were mated to normal tailed tufted homozygotes to observe recombination between T and tf . Parental types: short tail, non-tufted, T190, and normal tail, tufted. Recombinant types: short tail, tufted, and normal tail, non-tufted, T190.

have a generally high, but very variable, ratio whereas the reciprocal type have very high and quite invariant ratios. For comparison note that the parent $t^{w12}tf$ chromosome is also transmitted in very high proportion (the last six males of this type we have tested with progeny samples over 25 averaged 98% t -bearing offspring), with individual male ratios of 100, 100, 100, 99, 96 and 87% respectively. The parent Tt^{h17} chromosome, on the other hand, in similar samples, gave an average ratio of 65%, and individual males were more variable, having ratios of 76, 73, 73, 72, 61 and 42%. Thus, recombination between the $t^{w12}tf$ and Tt^{h17} chromosomes has altered the transmission ratio characteristic of the respective lethal factors, implying that some element(s), such as the 'A' factor predicted by Lyon¹⁰ to lie between T and t^{lethal} , has been switched by recombination. This explanation does not completely account for the data, however, as the Tt recombinant ratios are both generally higher and more variable from male to

male than those typical of Tt^{h17} . Further work on interactions between A factors and specific t -lethal factors will be necessary.

The possibility remained that the translocation inextricably associated with t^{h17} was in some mysterious way responsible for overcoming the recombination suppression of the complete t -haplotype $t^{w12}tf$. Preliminary data rule out the presence of T190 as a factor in overcoming the suppression: one of the chromosomes derived from the above cross, $Tt^{s6}tf$, genetically analysed to be $Tt^{w12}tf$ and now devoid of T190, was set up *trans* with the complete haplotypes $t^{w5}+$ and $t^{w32}+$. Recombination scored so far in these compound females is 2/25 for t^{w5} and 6/33 for t^{w32} , demonstrating that the presence of the translocation is not required for recombination.

We set out to test the hypothesis that recombination in t -haplotype/wild-type heterozygotes is impaired because of mismatching between normal chromatin and abnormal t -chromatin. In fact, as all t -haplotypes are thought to be derived from a common ancestral haplotype¹¹ with divergences due to mutation in recombinationally isolated chromatin, whatever is wrong with the DNA in one t -haplotype should be matchingly wrong in complementing but mutant t -DNA. Thus, it is not unreasonable that variant t -DNA should recombine with variant t -DNA. Therefore crossing-over should occur in long regions of homozygous abnormal t -chromatin. Our data show unequivocally that recombination suppression is indeed abrogated in such combinations. We conclude that recombination suppression is not due to some intrinsic inability of t -chromatin to undergo crossing-over. An alternative hypothesis suggested above and consistent with the data is that mismatching between normal and variant chromatins is responsible. DNA cloning should show whether the non-coding sequences of t -DNA are rearranged or scrambled with respect to wild type.

All the recombination events we measured occurred in a restricted region proximal to the tf marker. Our experimental design, which used two different t -haplotypes with the same $H-2$ haplotype, precluded studying recombination distal to *tufted*. Nevertheless, it is important to know that recombination suppression associated with t -haplotypes can be circumvented, and the use of second-generation recombinants with different $H-2$ types will make possible dissection of the t -region identifying the number of genes involved, and mapping them with respect to one another.

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Table 3 Transmission ratio of recombinant haplotypes

Haplotype	Male no.	Total informative offspring	% Of offspring carrying Tt or t -haplotype (ratio)
Tt^{s1}	1, 2	155	44
	3	127	28
Total		282	37
Tt^{s5}	1	63	64
	2, 3, 4	91	67
	5, 6	67	88
Total		221	72
Tt^{s6}	1	60	95
	2	119	89
	3	76	70
Total		255	84
Tt^{s10}	1, 2	110	51
	3	67	69
	4, 5, 6	88	69
	7	78	100
Total		343	70
Tt^{s11}	1	92	79
	2	69	35
Total		161	60
t^{s2}	1-2	9	100
t^{s3}	1-2	15	100
t^{s8}	1-7	146	88
t^{s9}	1-2	25	84
t^{s12}	1-6	83	84

Transmission ratio was measured in Tt haplotypes by mating $Tt/++$ males to $+/+$ females and scoring tails at birth. The ratio of Tt is calculated as short tail/total mice born. Occasionally the ratio of two or three brothers was measured collectively. The ratio of t -haplotypes was measured in two ways. First, $+/t$ males were mated to $T/+$ females, where the measure of the male ratio is tailless/short tailed plus tailless, as the normal tails are noninformative. Second, T/t males were mated to $+/+$ females and the ratio calculated as normal tail/total mice born. The data for the t -haplotypes are pooled within a haplotype because the numbers are scant because of semi-sterility due to the presence of T190.

BOOK REVIEWS

Séveso: the intrigue and the infighting

Alastair Hay

THE message of *The Superpoison* is both powerful and moving. The third book on dioxin to have been published recently, it covers ground similar to a previous one by Thomas Whiteside (for review see *Nature* 283, 229; 1980). Margerison, Wallace and Hallenstein, however, are specifically interested in the effects of dioxin on the residents of Séveso. This small Italian town fifteen miles north of Milan was contaminated by the inadvertent discharge of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (dioxin) when a reactor manufacturing 2, 4, 5-trichlorophenol overheated. Margerison *et al.* have written a well researched book on Séveso which deals with the subject at every level: human, scientific and political. Like Whiteside, they show that the Séveso residents were pawns caught in a process which rapidly lost its human element.

Confusion became a fact of life for the people in Séveso and the surrounding towns. Some leading scientists warned residents that dioxin was extremely dangerous; others, of equal repute, claimed there was no hazard. As the authors point out even the Catholic Church had to have its say. This was particularly true on the question of abortion brought into focus by the teratogenic properties of dioxin. When women, pregnant at the time of the reactor accident, were warned that exposure to dioxin might cause them to give birth to malformed children, the Vatican newspaper *Osservatore Romano* declared: "Is the mere possibility of the risk of malformation a sufficient reason to eliminate an unborn human being which undoubtedly has a greater chance of being born normal than abnormal?". Cardinal Colombo, Archbishop of Milan, joined the argument, according to Margerison and his co-authors, "by praising certain couples who had volunteered to adopt any malformed children born in the Séveso area, and appealing for others to come forward".

In the opinion of the authors it was the pregnant women from Séveso who had the most harrowing time. They claim that it was not only the church and politicians of a right-wing persuasion that made life difficult for the women, but that the Left were equally guilty in this regard, manipulating the situation at Séveso to advance their cause for abortion law reform in Italy. The campaign to reform the law and make abortion easier was

The Superpoison. By T. Margerison, M. Wallace and D. Hallenstein. Pp.236. (Macmillan, London: 1981.) £7.95.

eventually successful, but action came too late to help the women of Séveso. For many, the risk of having a malformed child was too great. The government claimed that in such cases abortion was legally available in Italy, the law making adequate provision. The reality was quite different. Most doctors refused to terminate pregnancies, subjecting the women to humiliating treatment. In the end only 34 pregnancies were terminated legally. At least twice that number are said to have been performed either abroad or illegally in Italy.

Controversy still rages in Italy over the effects of dioxin on the children born to mothers who decided against abortions. Three sources — the Italian Parliamentary Commission report on Séveso, the former head of the Health and Epidemiology Commission for Séveso, Professor Gaetano Fara, and Dr Guiseppe Reggiani, Director of Medical Research for Hoffmann-La Roche — all claim that there is no increase in the rate of malformation in the children born in Séveso. The authors of *The Superpoison* are not convinced. Acknowledging that the lack of statistics for malformation rates in Italy as a whole makes difficult the interpretation of the situation at Séveso, they nevertheless feel that there is "cause for serious concern".

Their concern is based on claims made by a local general practitioner working in Séveso. According to Alberto Colombi and the People's Scientific and Technical Committee — a largely left-wing association of doctors and scientists — there is gross under-reportage of the number of malformations. It is certainly true that the number of abnormalities is higher than official figures would suggest. The reason for their exclusion from published statistics, however, is explicable. Many of the malformations are minor. Their inclusion, although increasing the total number of cases, still does not alter the general pattern. Whichever set of figures is chosen and compared with the norm for various regions of Italy or with other countries in Western Europe, the answer is the same:

the rate for Séveso does not appear to be higher than anywhere else. However, research in progress may alter this conclusion.

The authors make much of the differences in the published figures for the number of cases of chloracne — the disfiguring skin disease caused by dioxin. In their view, "with so much disagreement between published figures, it is difficult to have confidence in the statistics" for the number of cases of chloracne observed. Again, the authors believe that the authorities are underestimating the seriousness of the situation and they cite various local doctors to support this charge. One such, the local Séveso health officer, Dr Francesco Uberti, is quoted as saying that he found 150 cases in children at one school alone. Uberti claims that the Milan regional authorities denied that his cases were genuine chloracne and "manipulated the facts". Diagnosis of chloracne can be difficult, as the authors admit, and this is at the basis of the disagreements about incidence. But this book was prepared some time ago, and the situation is now much clearer. A final figure for the total number of children who contracted chloracne has been agreed; it is 187.

Disagreements about the actions taken by the local authorities to protect the population at Séveso are well known. Margerison *et al.* insist that Givaudan and Hoffmann-La Roche, the principal companies involved in the dioxin problem, were aware of the dangers facing the residents of Séveso. They are unimpressed by the companies' arguments that it took time to construct a map of dioxin contamination preparatory to evacuating a section of the Séveso population. Indeed, they imply that the companies convinced the Italian authorities that the situation at Séveso was not dangerous. Two weeks after the dioxin discharge from the reactor, the authorities were indeed claiming that an evacuation was unnecessary. According to the authors, this complacency was shattered when Roche's Director of Medical Research insisted that an evacuation was absolutely essential. Many of these facts are not in dispute; however, the authors' thesis that the authorities were lulled into a sense of false security by Givaudan and Roche only to be caught out by Roche later on is not really proven.

All three authors are professional

journalists. Tom Margerison is a well known science journalist and this book has benefited from his skills. Technical details essential to the arguments are put clearly and simply. The operation of the trichlorophenol reactor at Séveso is well described. Accounts on the operation of the reactor differ, say the authors, depending on whether it is the workers themselves describing the operation or the management describing the instructions given to the workforce.

No satisfactory explanation is available yet to explain why the accident occurred. The authors find attractive a theory that there may have been sabotage. They say that one piece of evidence supports this. The reactor contents at Séveso are not alkaline as might have been expected. It is as though they were neutralized with acid, say the authors, giving credence to the sabotage theory. They do admit, however, that the pH change may be attributable to temperature differences, as indeed it is. Experiments designed to re-create the conditions in the Séveso reactor have since shown that at temperatures in excess of 260°C the pH of the contents changes from

alkaline to acid. The temperature of the reactor at Séveso is thought to have reached at least 350°C. Sabotage is ruled out in this case.

Political intrigue was rife at Séveso and this book describes it in ample detail. Scientific infighting also occurred and the authors take many irreverent swipes at the various experts involved with the dioxin problem. Interviews with many of the Séveso residents confirm that their treatment by the various authorities has been high handed. The authors succeed in showing that the residents at Séveso have had a raw deal. In their view the final immorality of the whole episode was that "Hoffmann-La Roche had the benefit of a profitable product while the innocent citizens of Séveso unknowingly took the risk". This may well be the verdict of history. But as more information accrues it is clear that the issue is not as clear cut as the authors make out. The companies certainly have much to answer for, but so too do the Italian authorities. □

Alastair Hay is a Lecturer in the Department of Chemical Pathology at the University of Leeds.

More from Harris

Alan E.H. Emery

The Principles of Human Biochemical Genetics. By Harry Harris. 3rd Edn. Pp.558. (Elsevier/North Holland Biomedical: 1980.) Hbk \$65.75, Dfl.135; pbk £26.75, Dfl.55.

THE first edition of this book, published in 1959, was an immediate success. The subject of human biochemical genetics was then in its infancy but there were sufficient pointers to indicate the exciting possibilities for the future. It has to be remembered that until Garrod introduced the idea of inherited disorders involving chemical processes (so-called inborn errors of metabolism) at the turn of the century, human genetics had been largely concerned with the inheritance of obvious structural abnormalities. In 1923 Garrod was only able to describe some half-dozen rare inborn errors of metabolism, but now Professor Harris details some 120 such disorders and a further 73 enzyme and protein polymorphisms.

This new edition is divided into nine chapters with an appendix listing details of specific enzyme deficiency disorders, and a similar appendix of enzyme and protein polymorphisms. The introductory chapter on gene mutations and single amino acid substitutions is, like the rest of the book, a model of clarity and could well form the basis of any graduate course in genetics. Genes can no longer be considered merely discrete sections of DNA contiguous with each other, but are frequently separated by intervening sequences where the relevant messenger RNA is not translated into protein. The function of these intervening sequences is as yet not clear, but in the future the unravelling of their role in gene action could well herald yet another new departure in biochemical genetics.

Two chapters are devoted to the principles of gene action which are largely, though not exclusively, illustrated from findings in the haemoglobinopathies. A discussion of gene localization (mapping) is also included. Until recently gene localization was dependent on the study of pedigree and somatic cell hybrids, but is now being greatly helped by the use of restriction endonucleases. In the near future this, too, is going to generate an entirely new perspective of gene structure and localization.

Subsequent chapters also deal with quantitative and qualitative variations in enzymes, inborn errors of metabolism and blood group substances. However many enzyme and protein variations (and the more recently discovered variations at the DNA level of restriction endonuclease cleavage sites) appear to be unrelated to any specific disorder, yet often their incidence is far greater than could be accounted for by mutation alone. Harris suggests that the term polymorphism might be reserved for those variations in which

Plasma physics for robust readers

A.D.R. Phelps

Plasma Physics for Nuclear Fusion. By Kenro Miyamoto. Pp.610. (MIT Press: 1980.) £35, \$50. *Fundamentals of Plasma Physics.* By V.E. Golant, A.P. Zhilinsky and I.E. Sakharov. Pp.405. (Wiley-Interscience: 1980.) £22.10, \$51.90.

BOTH of these books treat several aspects of plasma physics in detail, and yet neither provides a complete coverage of the subject — Golant *et al.* intentionally omit the important subject of waves in plasmas and Miyamoto, as implied by his title, concentrates on those topics in plasma physics which relate to nuclear fusion. Both are appropriate for postgraduate courses, and should appeal particularly to the mature research scientist.

The text by Miyamoto uses rationalized mks units and is a translation of his 1976 Japanese publication. Some opportunity appears to have been taken to update a few of the references, but some of the most recent results of nuclear fusion research have not been included. Nevertheless, much of the content has not hitherto existed in such digestible textbook form.

Professor Miyamoto divides his 16 chapters into four sections: fundamentals, which includes a review of fusion criteria, magnetic field configurations, single particle motion, Coulomb collisions and kinetic equations; MHD, covering equilibrium, diffusion and confinement, and instabilities; kinetic description of waves and instabilities, which treats waves

in cold and hot plasmas and velocity space instabilities; and finally, heating, diagnostics and confinement.

Fundamentals of Plasma Physics is also a translation (from the original 1977 Russian edition) and, like Miyamoto's book, more readable than might be expected. In ten chapters the authors cover collisions, kinetic equations, equilibrium plasmas, distribution functions, transport processes and magnetic field influences on particle motion, transport processes and plasma confinement. Cgs units are used. The notation is somewhat inconsistent in places, for example rot and curl appear on the same page and the Laplacian operator used in the text does not correspond to that given in the symbols list. Yet these are minor points which should not worry the relatively robust reader for whom both books are best suited.

Whereas Miyamoto's book essentially involves the high temperature collisionless type of plasma, Golant *et al.* address themselves more to gas discharges and ionization phenomena. Although there is some overlap in subject matter between the two books, the difference in emphasis makes them complementary. Thus one might expect the libraries of those research laboratories and universities engaged in plasma physics research to invest in both of them. □

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the commonest identifiable allele has a frequency no greater than 0.99 and therefore the proportion of heterozygotes is at least two per cent of the population. It is difficult to account for the high prevalence of such polymorphisms and the author presents a very readable account of the problem. It might, of course, turn out that most of these variations are no more than "random noise", but it is difficult to accept that this could be the entire explanation.

Altogether this is a fine book which can be unreservedly recommended as a graduate student text as well as a work of

reference. Of course, because of the enormous growth of the subject, this new edition might now appeal more to the specialist. Perhaps for a more general readership Professor Harris could be persuaded to produce a basic text similar to his *Introduction to Human Biochemical Genetics* which was published some 22 years ago by Cambridge University Press and which was the progenitor of the current text. □

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Where psychology meets biology

M. J. Morgan

Essay on Mind. By D. O. Hebb. Pp.159. (Lawrence Erlbaum Associates: 1980.) \$16.50.

IN THIS "essay", Professor Hebb expresses in clear and forceful language his conviction that "... mind and thought, consciousness and creativity and free will, are all biologically evident phenomena and seen most clearly in the light of evolutionary ideas". He summarizes the historical development of his own "cell assembly" theory, familiar from his classic book *The Organization of Behaviour* (Wiley, 1949) and to a certain extent attempts to relate it to modern developments in neurophysiology and psychology. There is little here that is very new, but Hebb was one of the first to argue that psychology is largely a biological subject, at a time when it was neither profitable nor popular. The gritty style of this book makes few concessions to the subtleties of professional philosophy, and somehow manages to convey the general impression that objections would be unwise and ill-informed, if not actually silly. Fortunately, no one need feel too offended by this brusque treatment, because the counter-arguments are put into the mouths of anonymous "writers", rather than being attributed to specific people.

On the heredity-environment issue Hebb neatly clears up some common confusions, and expresses the view that extreme environmentalism can have morally dangerous effects. It is pleasing to see the boot on the other foot for a change: usually it is environmentalists who assume the mantle of moral superiority. Returning to a theme familiar from his *Textbook* (W. B. Saunders, 1972) Hebb warns the young student that if he has an inherited predisposition to alcoholism, he had better recognize it quickly and take appropriate avoiding action. This he is not likely to do "... if he takes it for granted that all men are born equal ... and seeing others drink freely without becoming drunks,

takes it for granted that he can do the same". This is probably good advice, certainly no worse than that given by people who think that it is wicked to believe in genes.

The "cell-assembly" model can now be seen as one of the first "feature analysis" models of perception. Its essence is that cells or groups of cells coding for distinctive features such as lines are grouped together into superordinate assemblies by associative learning. These assemblies can in turn be grouped into assemblies representing concepts, and so on, presumably up to "grandmother assemblies", which would be activated when we were thinking of a particular person. Hebb notes that the physiological work of Hubel and Wiesel has given considerable support to this idea since he first produced it, but he quotes their hierarchical model as if it were established fact, without mentioning important criticisms. He also attempts to extend the theory in a way that many readers will probably find frankly metaphorical: we read, for example, that "Emotion is a disruption of cell assemblies".

The experiments that Hebb pioneered at McGill on effects of early environment in animals are given prominence and still raise issues of great interest. But as in the case of the discussions of visual perception, it is disappointing not to be presented with any more recent data, particularly those concerning effects of early environment on synaptic connections.

Possibly the most interesting aspect of this book is the historical insight it gives into the process by which Hebb arrived at his theory at a time when radical behaviourism made any physiological theorizing disreputable. That many of these early battles will now seem a bit outdated to the younger generation of psychologists is a measure of Hebb's success in helping to win them. □

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Clearing the decks

Adrian Friday

Comparative Biology and Evolutionary Relationships of Tree Shrews. Edited by W.P. Luckett. Pp.314. (Plenum: 1980.) \$39.50, £24.89.

TREE shrews are placental mammals of Southeast Asia. Although not rodents, they resemble squirrels and the Malay word "tupai" is used for both. Of all placental mammals, tree shrews are probably the most enigmatic and differences of opinion concerning their evolutionary relationships



The pen-tailed tree shrew, *Ptilocercus lowii*, from the plate in the original description by J.E. Gray.

have caused some heated exchanges among mammalogists. Originally included in that ragbag of an order, Insectivora (within which they had been particularly allied with the elephant shrews of Africa), the tree shrews came to be widely regarded as the least-specialized grade of primate largely as a result of the work of Alberta Carlsson and W. E. Le Gros Clark. In this volume there are contributions on the tree shrew skeleton, dentition and cranial circulation, their meagre fossil record, their nervous and reproductive systems, and the molecular evidence for their relationships. Behavioural studies on tree shrews are not represented: distinguished work in this area has already been published, but Luckett implies that it has little to contribute to solving the problem of tree shrew relationships.

Contributors to the volume were asked to consider two questions in their accounts: first, do tree shrews and primates share unique derived characters suggesting an immediate common ancestry; second, are there reasons for supposing that tree shrews, primates, bats and colugos ("flying lemurs") together form a discrete evolutionary group (Archonta) amongst mammals?

It was part of the message from the work

of Van Valen and of Martin in the late 1960s that progress in assessing the evolutionary affinities of the tree shrews and other problematic groups, collected together in the past as Insectivora, would be made only if comparisons were made between these animals and all other mammalian orders. However, if Luckett had insisted on such application from all his authors, several of them would have been consigned to a lifetime's work.

The fascination of tree shrews reaches beyond biologists interested in Insectivora (in the widest sense) to include those concerned with the principles of phylogenetic reconstruction. In his introductory chapter, Luckett succinctly summarizes the phylogenetic methods described by Hennig, and uses them to dispose of the early arguments for immediate common ancestry shared by tree shrews and elephant shrews. Particular emphasis is given to the reconstruction of the primitive state for various characters in the hypothetical eutherian common

ancestor. In reviewing the evidence of all the contributors, Luckett reaffirms Butler's and McDowell's concept of the isolation of both the tree shrews and the elephant shrews, leaving other Insectivora as an assemblage of animals which may have some claim to monophyly.

It is hardly surprising that there are few firm conclusions concerning the relationships of the tree shrews, although Szalay and Drawhorn reassert the validity of Archonta, a tactic supported in varying degrees by the two chapters on molecular evidence. Inevitably, much recent work on tree shrews has been inconclusive, even destructive, but this might be viewed as a clearing of the decks. In any case, a volume that includes several chapters of thoroughly admirable compilation and evaluation of morphological data is very welcome for that alone. □

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Pedagogical overview of quantum fields

Paul Davies

Quantum Field Theory. By Claude Itzykson and Jean-Bernard Zuber. Pp.700. (McGraw-Hill: 1980.) £26.95, \$55.50.

A NEW generation of textbooks on quantum field theory is appearing, and this monumental work of 700 pages promises to become the standard book to replace Schweber's *An Introduction to Relativistic Quantum Field Theory* (Harper & Row, 1961), or Bjorken and Drell's *Relativistic Quantum Mechanics* (McGraw Hill, 1964) and *Relativistic Quantum Fields* (McGraw Hill, 1965), both nearly 20 years old. The subject has moved a long way in that time, and a comprehensive, pedagogical book covering both the traditional material and modern developments is long overdue.

The authors have been lavish and ambitious in their approach. Starting with classical field theory, they build upon an extensive discussion of the Dirac equation towards a thorough treatment of conventional quantum electrodynamics. On the way, all the mathematical formalism needed for the more familiar applications is presented. Also included are some topics that are generally hard to find in textbooks: the Klein paradox, the Heisenberg-Euler effective action, the Fock-Schwinger proper time method, finite temperature states, massive vector fields and the Casimir vacuum force effect. Especially welcome are the treatments of symmetries, the PCT and spin-statistics theorems, and a section on Grassmann algebra.

All the routine machinery of interacting

relativistic quantum fields then emerge via the S-matrix and Feynman rules, with extensive applications to quantum electrodynamic processes on the way. Then, on through dispersion relations and analyticity to an extensive section on radiative corrections and renormalization theory. Once again, one welcomes the inclusion of modern topics like Wick rotation, asymptotic behaviour and dimensional regularization.

But all this comprises only the first half of the book! The really attractive feature is the comprehensive treatment of recent functional methods, gauge theories and asymptotics which occupies the last 300 pages. An extensive discussion of non-Abelian gauge fields and spontaneous symmetry breaking opens the way to topics such as the Weinberg-Salam theory, anomalies, Fadeev-Popov ghosts and scaling behaviour in scattering theory.

This valuable survey is written with meticulous care and thoroughness. Although intended primarily for graduate students, there is such a wealth of material that undergraduates will find it useful for reference. Those learning relativistic quantum mechanics will also benefit from the chapter on the Dirac equation, hole theory and hydrogen-like atoms. Certainly, any student of quantum field theory will find everything necessary for a thorough grounding in this increasingly important subject. □

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